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(54) Title: COMPOUNDS AND METHODS TO TREAT INFECTIOUS DISEASES

(57) Abstract

There is disclosed pharmaccutical compositions that possess anti–infective activity against HIV virus and certain parasites. The pharmaceutical compositions comprise oral formulations having an active arylene bis (methyl carbonyl) derivative.

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COMPOUNDS AND METHODS TO TREAT INFECTIOUS DISEASES

Technical Field of the Invention

The present invention provides a class of compounds that react, under physiologic conditions, with proteins having adjacent or neighboring lysines. The compounds of the invention can also be used to treat infectious diseases such as HIV infection and malaria.

Background of the Invention

Nuclear importation is determined by the presence of a short sequence, called a nuclear localization signal (NLS), which functions relatively independently of its position relative to the remainder of the structure of object that is imported. In eukaryotic cells all proteins are made in the cytoplasm. In general, those proteins larger than 40 kD, that are specifically localized in the nucleus of the cell, must be actively imported into the nucleus through the nuclear membrane from the cytoplasm via an ATP-dependent mechanism that is independent of cell division. The proteins, and nucleic acids, that are imported have a nuclear localization signal (NLS), usually located within the NH_2 terminal segment of the protein. Several such sequences are known, including, for example, (a) PKKKRKV from large T antigen of SV40 (Kalderon et al., 1984, *Cell* 39:499-509, 1984); (b) [AV]KRPAATKKAGQAKKKK[LD] from nucleoplasmin, in which only one of the two bracketed sequences is required (Dingwall et al., *J. Cell Biol.* 107:841-49, 1988); (c) PRRRRSQS from hepatitis B HbcAg-; (d) KRSAEGGNPPKPLKKLR from the retinoblastoma gene product $p110^{rbl}$ (Zacksenhaus et al., *Mol. Cell. Biol.* 13:4588, 1993); and (e) KIRLPRGGKKKYKLK from the matrix protein of HIV-1, Bukrinsky et al., *Nature* 365:666, 1993).

The primary structure of the HIV NLS contains consecutive lysines called the N^c moieties. The cellular proteins or protein complexes that recognize and transport proteins bearing NLS sequences are incompletely understood. It appears that there are proteins of the cytoplasmic face of the nuclear membrane that recognize the NLS and, after such recognition, it is this complex that is transported through the nuclear pore complex. (Stochaj et al., *Eur. J. Cell Biol.* 59:1-11, 1992; Hurt, *FEBS Letters* 325:76-80, 1993; Pante et al., 1993, *J. Cell. Biol.* 122:977-84, 1993; and Forbes, *Ann. Rev. Cell Biol.* 8:495-527, 1992).

A receptor for the NLS sequence has been described in a *Xenopus* system (Görlich, *Cell* 79:767, 1994). It is a cytoplasmic 60 kDa protein which is homologous with previously described proteins of unknown function, SRP1p of yeast (Yano et al., *Mol. Cell. Biol.* 12:5640, 1992) and Rch1 of mammals (Cuomo, *Proc. Natl. Acad. Sci. USA* 91:6156, 1994). Two inhibitors of the nuclear localization process have been described, including lectins (*e.g.*, wheat germ agglutinin (WGA)) that bind to the O-linked glycoproteins associated with nuclear localization (Dabauvalle, *Exp. Cell Res.* 174:291-96, 1988; and Sterne-Marr et al., *J.*

Cell Biol. 116:271, 1992) and (g-S)GTP, a non-hydrolyzable analog of GTP (Melchior, *J. Cell Biol.* 123:1649, 1993). However, neither (g-S)GTP nor WGA can be used as pharmaceuticals because proteins and thiotriphosphates cannot access intracellular sites of action. Further, GTPases are involved in a multitude of cell processes and intercellular signaling, thus, the use of a general inhibitor of GTPases would likely lead to unacceptable side effects.

Although HIV-1 is a retrovirus, it and other lentiviruses must be distinguished from viruses of the onco-retrovirus group, which are not associated with progressive fatal infection. For example, lentiviruses replicate in non-proliferating cells, such as terminally differentiated macrophages (Weinberg, *J. Exp. Med.* 172:1477-82, 1991) while onco-retroviruses, do not Humphries and Temin, *J. Virol.* 14:531-46, 1974). Lentiviruses are able to maintain themselves in a non-integrated, extrachromosomal form in resting T-cells (Stevenson et al., *EMBO J.* 9:1551-60, 1990; Bukrinsky et al., *Science* 254:423, 1991; and Zack et al., *J. Virol.* 66:1717-25, 1992).

The productive infection of a cell by a retroviruses involves the steps of penetration into the cell, synthesis of a DNA genome from the RNA genetic material in the virion and insertion of the DNA genome into a chromosome of the host, thereby forming a provirus. Both lenti- and oncoretroviruses gain access to the host cell's nucleus during mitosis when the nuclear membrane dissolves. However, the lentiviruses are also able to cross the nuclear membrane because viral proteins containing nuclear localization sequences are associated with the viral nucleoprotein complex. The productive infection of terminally differentiated macrophages located in the central nervous system is thought to be responsible for the dementia associated with AIDS (Keonig, S., et al., *Science* 233:1089, 1986; Wiley et al., *Proc. Natl. Acad. Sci. USA* 83:7089-93, 1986; and Price, R.W., et al., *Science* 239:586-92, 1988). The infection of terminally differentiated macrophages in the lymphoid system is known to cause aberrant cytokine production (Guilian et al., *Science* 250:1593, 1990; and Fauci et al., *Ann. Int. Med.* 114:678, 1991). Thus, the wasting syndrome associated with HIV-1, also known as "slim" disease, is believed to be a pathological process that is independent of the loss of CD4-T-cells. Rather, the pathobiology of the wasting is closely related to the pathobiology of cachexia in chronic inflammatory and malignant diseases (Weiss, *Science* 260:1273, 1993). For these reasons, the inhibition on HIV-1 infection of macrophages and other non-dividing cells is a highly desired modality in the treatment of HIV-1 infection, especially for patients wherein dementia or cachexia dominate the clinical picture.

Macrophages play an important role in the transmission of HIV as well. During early stages of the infection, macrophages and cells of the macrophage lineage (*i.e.*, dendritic cells) may be the primary reservoir of HIV-1 in the body, supporting infection of T cells by antigen presentation activities (Pantaleo et al., *Nature* 362:355-358, 1993) as well as via the release of free virus.

It is should be noted that macrophage-tropic strains of HIV-1 predominate in the early stages of infection. Thus, it appears that the infection of macrophages is particularly important during the development of a chronic infective state of the host in a newly infected subject. Secondly, macrophages are the HIV-susceptible cell type most readily passed during sexual intercourse from an HIV-infected individual into the circulation of an uninfected individual.

Finally, infection of quiescent T cells by HIV-1 has been shown to take place *in vitro* (Stevenson et al., *EMBO J.* 9:1551-1560, 1990; Zack, *Cell* 61:213-222, 1990) and probably constitutes an important pathway for the spread of infection *in vivo* at various stages of the disease (Bukrinsky et al., *Science* 254:423-427, 1991). Although HIV-1 does not establish productive replication in quiescent T cells, the extrachromosomal retroviral DNA can persist in the cytoplasm of such cells for a considerable period of time, and initiate replication upon activation of the host cell (Stevenson et al., *EMBO J.* 9:1551-1560, 1990; Spina et al., *J. Exp. Med.* 179:115-123, 1994; and Miller et al., *J. Exp. Med.* 179:101-113, 1994). Thus, there is a need to prevent infection of macrophages by HIV.

The function of the pre-integration complex of retrovirus is to gain access to nucleus (Brown et al., *Cell* 49:347, 1987). Onco-retroviruses gain access to the nucleus upon the dissolution of the nuclear membrane in mitosis. By contrast, lentiviruses contain two distinct proteins that mediate nuclear access through the nuclear pore complex in the absence of cellular division. For the first of these, the matrix protein (MA or p17), nuclear importation activity is clearly due to the presence of a trilysyl-containing NLS sequence (Bukrinsky et al., *Nature* 365:666, 1993; and von Schwedler et al., *Proc. Natl. Acad. Sci. USA* 91:6992, 1994). Vpr is a protein that is part of a preintegration complex, but does not contain an identifiable NLS consensus sequence (Emerman et al., *Nature* 369:108, 1994; and Heinzinger et al., *Proc. Natl. Acad. Sci. USA* 91:7311, 1994).

Treatment of an infectious disease with chemicals involves killing the infectious agent or inhibiting growth of the infectious agent. Humans, domestic pets and livestocks are hosts to a variety of parasites. Parasites do not comprise a single taxonomic group, but are found within the protozoans and metazoans, among other groups. In many ways, infectious parasitic diseases resemble infectious diseases caused by microbiologicals such as fungi, bacteria and viruses. Malaria remains one of the major health problems in tropical climates. It is estimated that 300 million people a year are infected with malaria (World Health Organization, 1990, Malaria pp.15-27. In Tropical Diseases, Progress in Research 1989-1990, Geneva). Malaria is transmitted by Anopheles mosquitoes in endemic areas, and often by blood transfusion in eradicated areas.

Malaria in humans is caused by at least four protozoan species of Plasmodium: *P. falciparum, P. vivax, P. ovale and P. malariae*. The asexual erythrocytic parasite, merozoite, is the stage in the life cycle that causes the pathology of malaria with a characteristic pattern of fever, chills and sweats. Anemia, acute renal failure and disturbances in consciousness are

often associated with malarial infection. *P. falciparum* can produce a large number of parasites in blood rapidly, and causes the most morbidity and mortality.

The most important treatment of malaria to date is chemotherapy using a number of natural and synthetic drugs. Antifolates, such as pyrimethamine, inhibit the parasite's dihydrofolate reductase, whereas the aminoquinolines, such as chloroquine (4-aminoquinoline) have the digestive vacuoles as their major site of action. Prior to the introduction of chloroquine in the 1940's, quinine was the only effective drug for treatment of malaria. Chloroquine is commonly used to treat acute infections with all four species, but has no effect on relapses of infection by *P. vivax or P. ovale*. Chloroquine (500 mg weekly) may also be used to prevent malaria by suppressing the stages that multiply in the erythrocytes and cause the symptoms.

However, the use of these drugs in certain areas and in the future will be seriously hampered by the emergence of drug resistant parasites. Chloroquine resistance is widespread and will continue to appear in new areas.

Summary of the Invention

The invention involves a class of aryl alkyl carbonyl compounds, particularly, divalent aryl carbonyl moieties N-linked through the arene to a nitrogen-containing heterocyclic functionality, e.g., an acetyl or propanoyl substituted aniline moiety N-linked to a pyrimidinium, pyrimidine or triazine moiety. The invention further encompasses methods of using the compounds of the invention to form tandem Schiff bases in proteins having neighboring N^{ε} moieties of lysine residues. As used, herein, neighboring N^{ε} moieties are two N^{ε} moieties of a protein that approach each other as close as the carbonyls of the arylene bis (methyl carbonyl) compounds of the invention, when the protein is in its natured conformation. As used herein neighboring, adjacent and juxtaposed are equivalent terms in reference to N^{ε} moieties and refer to the physical locations of the N^{ε} moieties in the structure of the native protein and not to the positions of the lysines in the linear sequence.

The invention further encompasses methods of inhibiting productive infection by HIV-1 of terminally differentiated (non-dividing cells), particularly macrophages, by inhibition of the importation of the cytoplasmic HIV-1 complex into the nucleus of cell. Particularly the invention concerns the direct introduction across the cytoplasm membrane of a cell of compounds that block such importation. Thus, in one embodiment, the invention encompasses methods of using the above-described compounds to prevent productive infection of terminally differentiated macrophages and resting T-cells in HIV-1 infected subjects. Without limitation as to theory, the invention is believed to block the HIV-1 replication by the formation of tandem Schiff bases with neighboring N^{ε} moieties of viral proteins, a consequence of which is that the viral nucleoprotein complex does not pass across the nuclear membrane via interaction with the nuclear pore transport complex and/or other cellular components.

The invention further encompasses methods of using the compounds of the invention in treating or preventing infectious diseases such as those caused by parasites, particularly Plasmodium species that cause malaria.

Brief Description of the Drawings

Figures 1A-C show structures of exemplary compounds 2, 11 and 13, respectively.

Figures 2A-C show the effect of various concentrations of compound 2 on reverse transcriptase (RT) activity in the supernatant of HIV-1-infected monocytes. Specifically, Figure 2A shows Multiplicity of Infection (MOI) 1 ng p24 / 10^6 monocytes, cultured in presence of M-CSF, Figure 2B shows MOI 8 ng p24 / 10^6 monocytes, cultured in absence of M-CSF, and Figure 2C shows MOI 0.8 ng p24 / 10^6 monocytes, cultured in absence of M-CSF.

Figure 3 shows the effect of various concentrations of compound 2 on RT activity in supernatants of HIV-1-infected, mitogen-stimulated peripheral blood leukocytes, infected at 10 and 1.0 ng p24 / 10^6 cells, (Figure 3A and 3B) respectively.

Figure 4A-F shows the structures of exemplary compounds. Specifically, Figure 4A shows 2-amino-4-(3,5-diacetylphenyl)amino-1,6-dimethylpyrimidinium chloride (compound 2 or CNI-0294), Figure 4B shows 2-amino-4-(3,5-diacetylphenyl)amino-6-methylpyrimidine (compound 11 or CNI-1194), Figure 4Cshows 2-amino-4-(3-acetylphenyl)amino-6-methylpyrimidine (compound 15 or CNI-1594), Figure 4Dshows 2-amino-4-(4-acetylphenyl)amino-6-methylpyrimidine (compound 17 or CNI-1794), Figure 4E shows 3.5-diacetylaniline (compound 18 or CNI-1894), and Figure 4Fshows 4-phenylamino-2-amino-6-methylpyrimidine (compound 45 or CNI-4594).

Figure 5shows representative plasma concentrations over time in mice treated with compound 11. Female ND4 Swiss-Webster mice were given a single 50 mg/kg injection intraperitoneally (circles) or orally (squares). The calculated plasma concentrations, in mg/ml, was then plotted against the time of sampling.

Figures 6A-6B show chromatograms of plasma extracts from animals treated with compounds 2 or 15. Female ND4 Swiss-Webster mice were given a single i.p. injection of 50 mg/kg compound 2 (A) or 20 mg/kg compound 15 (B). The chromatogram shown for compound 2 was from the 2 hr time point, and that for compound 15 for the 1 hr time point. The peaks labeled "2" and "15" are the parent peaks for compound 2 and compound 15, respectively. The other peaks in the chromatogram represent possible metabolites (labeled "x") and endogenous plasma peaks.

Figures 7A-7D show the *in vitro* metabolism of the CNI compounds. The drugs were incubated with mouse liver post-mitochondrial supernatants and NADPH for various lengths of time. The chromatograms shown are from the 60 min time point for (A) compound 2, (B) compound 11, (C) compound 15, and (D) compound 18. The peaks labeled "2, 11, 15, 18" refer to the parent compound peaks, and those labeled "a-n" to putative metabolite peaks that

increased over time and were not present in control incubations. All off-scale peaks were single peaks, and the scale was chosen to allow presentation of trace metabolite peaks.

Figures 8A-8D show the *in vivo* metabolism of the exemplary compounds. Female ND4 Swiss Webster mice received a single intraperitoneal dose of (A) 50 mg/kg compound 2, (B) 50 mg/kg compound 11, (C) 20 mg/kg compound 15, or (D) 50 mg/kg compound 18. In all four graphs, the open bar represents the peak area of the parent compound and the black bars the apparent metabolite peaks. The metabolite peaks shown are (from left to right in each graph): (a) peak "d" (see Figure 7 for letter-designated peaks), peak "a", peak "c", and a peak eluting at 13 minutes; (b) peak "h", peak "e", peak "f", peak "g", a peak eluting at 14 minutes, and a peak eluting at 23 minutes; (C) peak "j", peak "i", peak "l", and a peak eluting at 14 minutes; (D) peak "m", peak "n", and a peak eluting at 11 minutes. The peak area units are arbitrary and calculated by the HPLC operating system.

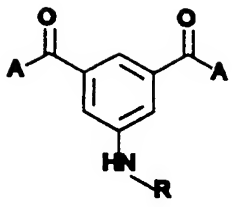
Figure 9 shows the activity of compound 2 against *Plasmodium berghei* infected mice. Female ND4 Swiss Webster mice were infected with infected erythrocytes and then treated once daily, for four days, with 50 mg/kg compound 2, or with distilled water. Six hours after the last dose, thin blood smears were made from each of the animals and the parasitemia was determined. The bars represent the median parasitemia (n=4 for controls and n=5 for treated).

Detailed Description of the Invention

Compounds

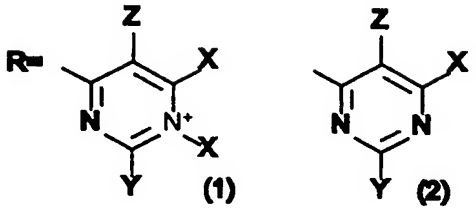
The compounds of the present invention can be synthesized by reacting aniline to form a compound of formula II, described below, wherein P is 0 or an acetyl or propanoyl derivative of aniline to form a compound of formula II; wherein P is 1 or a diacetyl or dipropanoyl derivative of aniline to form a compound of formula I or formula II; or wherein P is 2 with a chloro derivative of purine, aminomethylpyrimidine, diamino-triazine, or with a cyanoguanidine. The reaction can be performed at 90-100 °C in an aqueous solvent in the presence of a mineral acid to yield the corresponding aminophenyl pyridine or triazine. The pyrimidinium can be synthesized from the pyrimidine by reaction with an excess methyl iodide at 40-45 °C under reflux conditions in 1:1 acetonitrile/tetrahydrofuran or in a 1:1:2 mixture of dichloromethane/acetonitrile/tetrahydrofuran.

In a preferred embodiment the compounds of the invention are bis ketone arylene compounds having a third nitrogenous substituent. The nitrogenous substituent can be further substituted with an aromatic nitrogen-containing heterocyclic compound. The compounds of the invention are formed according to the formula (I):



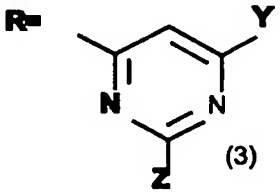
(I)

wherein A = CH_3 or CH_2CH_3, and

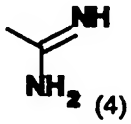


(1) (2)

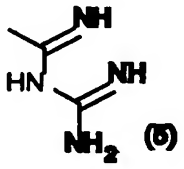
wherein X = NH_2, CH_3 or CH_2CH_3; X' = CH_3 or CH_2CH_3; Y = NH_2, $NHCH_3$, $N(CH_3)_2$; and Z = H, CH_3 or CH_2CH_3; or



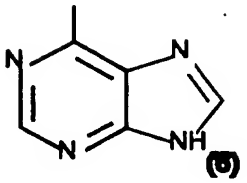
(3)



(4)



(5)



(6)

wherein Y' and Z', independently, = H, NH_2, $NHCH_3$, $N(CH_3)_2$ or $N^+(CH_3)_3$; and salts thereof.

Therapeutic Activity

A quantitative measurement of the activity of the compounds of the invention to block the replication of HIV-1 in non-dividing cells can be determined by culture of a macrophage-tropic strain of HIV-1 on peripheral blood-derived macrophages. The cells are cultured for 5-6 days prior to infection in a medium consisting of DMEM supplemented with 10% type A/B human serum and 200 U/ml Macrophage Colony Stimulating Factor, with half the medium changed after 3 days, to reach a density of about 10^6 cells per 5 ml well. A macrophage-

tropic viral stock may be grown on these cells. The concentration of infectious particles in the stock is estimated by measurement of p24 antigen concentration.

To test the effect of compounds of the invention on HIV-1 infection in the above-described culture system, the medium is removed and replaced with medium containing HIV-1 at a concentration of 1 ng of p24 (10^4 $TCID_{50}$ / ml (TCID= tissue culture infectious doses)) and a known concentration of the compound of the invention (the inhibitor). After 24 hours, the cultures were washed to remove non-adherent virus and the culture was re-fed with medium containing a compound at a desired concentration. The amount of replication of HIV-1 is estimated by an assay of the reverse transcriptase (RT) activity or by an assay of the concentration of p24 antigen in the culture medium every 2-3 days throughout the post-infection period. Repetition at various levels of inhibitor allows for the calculation of the concentration of inhibitor that achieves 50% inhibition of viral growth, IC_{50}. Table 1 discloses the IC_{50} of various inhibitors.

Table 1

Compound	IC_{50}
2-amino-4-(3,5-diacetylphenyl)amino-1,6-dimethylpyrimidinium iodide (Compound 2)	1 nM
2-amino-4-(3-acetylphenyl)amino-1,6-dimethylpyrimidinium iodide (Compound 14)	10 nM
2-amino-4-(3,5-diacetylphenyl)amino-6-methylpyrimidine (Compound 11)	50 nM
4-(3-acetylphenyl)amino-2-amino-6-methylpyrimidine (Compound No. 15)	15 nM

Alternatively, compounds may all be compared for inhibition of HIV replication at a fixed concentration. Table 2 shows compounds used at a concentration of 100 nM to inhibit the production of HIV-1 in cultured monocytes infected with HIV-1 10 days prior to assay (10 ng of p24/ 10^6 cells). The production of HIV-1 in each treated culture is reported as percentage of untreated control.

Table 2

Compound	Viral Production
N-(3,5-diacetylphenyl)biguanide hydrochloride (12)	12%
2-(3,5-diacetylphenyl)amino-4,6-diamino-1,3,5-triazine (13)	14%
4-(3-acetylphenyl)amino-2-amino-6-methylpyrimidine (17)	20%
3,5-diacetylaniline	20%
N,N-dimethyl-3,5-diacetylaniline	25%
2,6-diacetylaniline	28%
3,5-diacetylpyridine	58%

Figure 2A presents further results for compound 2 blocking replication of HIV-1 in

purified monocytes, cultured in medium supplemented with monocyte-colony stimulating factor (M-CSF). The cultures were treated with none or between 10^{-12} and 10^{-6} M compound 2 and, simultaneously with the beginning of treatment, the cells were exposed to the monocyte-tropic strain HIV-1_{ADA} at about 0.01 $TCID_{50}$/cell (1 ng p24/10^6 cells) for 2 hours. Samples were withdrawn at days 3, 6, 10, 14 and 17 after infection and assayed for reverse transcription activity. The results show that under these conditions the IC_{50} concentrations is between 0.1 and 1.0 nM and that a concentration of between 0.1 μM and 1.0 μM completely inhibits the replication of the virus.

Figures 2B and 2C show the effects of various concentrations of compound 2 on the production of HIV-1 in monocyte cultures not supplemented with M-CSF. In these studies, MOI (determined by concentration of p24 antigen) was; Figure 2B (8 ng/10^6 cells) and Figure 2C (0.8 ng/10^6 cells). These experiments showed IC_{50}s of about 10 nM and of less than 1.0 nM respectively. However, despite a more potent *in vitro* therapeutic activity, compound 2 did not provide significant plasma concentrations when administered orally.

The inhibition of the replication of HIV-1 was not due to general cytotoxic effects of the compound. Concentrations (*in vitro*) of compound 2 as high as 10 μM were without toxic effects on the monocyte cultures, as determined by lactate dehydrogenase release and trypan blue exclusion. Compound 2 provided also specific inhibitory activity (Figures 3A and 3B) because mitogen-stimulated peripheral blood leukocytes were cultured in IL-2 supplemented medium and were exposed to the HIV-1_{ADA} at p24 concentrations of 10 and 1 ng/10^6 cells, respectively. In this experiment up to 10 μM compound 2 had only a marginal effect on viral production at the higher MOI. At the lower MOI, 1 and 10 μM of compound 2 caused an approximate 2-fold reduction in viral output.

Inhibition of HIV-1 importation into the nucleus of non-dividing cells can be measured directly. MT-4 cells were growth arrested by treatment with aphidicolin and exposed to HIV-1. PCR amplification was used to detect double-stranded closed circular HIV-1 genomes, which were formed only after nuclear importation, by selecting primers that bridge the junction point of the genome.

The present invention provides a method for treating HIV-1 infection by oral administering to an HIV-1-infected subject an oral pharmaceutical composition having, as an active ingredient, an effective amount of a compound of formula (I). In one embodiment the compound to be administered is compound 11 (Figure 1).

Oral Pharmaceutical Formulations

The compounds of the present invention can be used to treat patients infected with HIV virus and can be used to treat patients having parasitic diseases, and in particular malaria. Such a compound can be administered to a patient either by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s).

The compounds can be formulated readily using pharmaceutically acceptable carriers and buffers well-known in the art into dosages suitable for oral administration. Such carriers

enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Toxicology and Pharmacokinetics

Compounds 2 and 11 were studied for their toxicology and suitability for oral administration, which is a necessary and preferred route of administration for treating HIV infection and for malaria where the means for parenteral delivery may not be available. Both compounds were tested for their ability to inhibit CFU-GM (granulocyte macrophage colony forming units) with continuous exposure and compared to AZT (a known reverse transcriptase inhibitor). The results showed similar toxicity profile to AZT and should not se

a bone marrow toxicity problem upon *in vivo* administration.

In addition, compound 11 was tested for anti-viral HIV activity (measured by RT activity) alone or in combination with another RT inhibitor, 3TC. The results showed that the combination of both drugs provided RT inhibition that was more-than-additive, or clearly synergistic. Doses of from 0.1 to 10 μM of compound 11 and from 1-100 nM of 3TC were tested. These data (plus additional data in combination with AZT provided herein) indicate that compound 11 and similar compounds that inhibit entry of the preintegration complex into the nucleus are effective in combination with other anti-HIV therapies, such as reverse transcriptase inhibitors and HIV protease inhibitors.

Compounds 2 and 11 were compared for oral bioavailability in mice and rats. Compound 11 had about a 2 hour plasma half life in rats and a t ½ decay of 96 min, 30 min and 26 min at 4, 24 and 37 °C, respectively. Compound 2 had a much longer half life (more than 4 hours in mice blood) and a t ½ decay of greater than 4 hours at 4, 24 and 37 °C. Compound 11 was tested for *in vitro* metabolism in mice liver S9 (around 30% parent metabolized), in rat liver S9 (less than 10% metabolized), in monkey liver S9 (most of parent metabolized) and in human liver S9 (less than 10% metabolized). Compound 2 was tested for *in vitro* metabolism in mice liver S9 (around 30% parent metabolized), in rat liver S9 (less than 10% metabolized), in monkey liver S9 (around 50% of parent metabolized) and in human liver S9 (less than 10% metabolized).

The following table lists the oral bioavailability of compounds 2 and 11 in mice and rats.

Administration	cpd 11 mice	cpd 11 rat	cpd 2 mice	cpd 2 rat
dosage mg/kg	50	50	50	50
Tmax	11.65	30.0	nd	20.61
Cmax ug/ml	1.00	0.55		0.21
T ½ absorption, min	7.87			
T ½ elimination, min	8.29			8.29
AUC (ug/ml/min)	31.57	120.30		12.01
bioavialability (%)	29.78	44.29	0	2.95
modeling (R2)	0.9204	NCP		0.94

NCP is calculated by a non-compartmental model.
nd is no detection of parent compound.

These data show that similar aqueous formulations of compounds 2 and 11 show the superior bioavailability of compound 11 to achieve and maintain needed and active plasma concentrations of drug with oral administration. Compound 11 showed significant bioavailability in both the rat (44%) and mice (29%) at an oral dose of 50 mg/kg. Neither compound showed a significant first pass effect in human liver S9 (<10%) but both compounds were metabolized by monkey liver S9 significantly.

Derivatizing Proteins

The compounds of the present invention of formula II, wherein P is 1 or 2. can be used to derivatize a target protein and thereby determine the presence of adjacent N^{ε}-moieties. The test reaction can be conducted in aqueous buffer at mild to moderate alkaline pH, between about 7.2 and 8.0. Specific derivatization of the target protein can be detected by any means that separates protein-bound and free derivatizing compound. The derivatizing compound optionally can be detected by radiolabeling it. In one embodiment, the compound can be synthesized using ^{14}C-methyliodide in place of methyliodide. Alternatively, use can be made of the strong UV absorption or fluorescence of the derivatizing compounds. Compound 2, for example, has a absorption peak of 16,000 M^{-1} cm^{-1} at l=298 nm. In a preferred embodiment the target protein is derivatized by a compound of the invention, irreversibly reduced with sodium borohydride or cyanoborohydride and fragmented into peptides by trypsin or the like. The resultant peptides can be compared with the peptides obtained from an unreacted sample of the protein by analysis using any chromatographic or electrophoretic technique that resolves peptides, such as, reverse phase High Performance Liquid Chromatography (HPLC). When the peptides are resolved by any high resolution chromatography procedure, the derivatized peptides can be detected by their altered elution time and the absorbance at l=298 nm.

Preferably, the reaction is conducted at various pH points to determine whether a positive result can be obtained at any point within the expected range. A positive result, i.e., a result that indicates the presence of adjacent N^{ε} moieties, is one in which a large fraction of each of a limited number (between 1-4) of peptides of the target protein are derivatized and negligible amounts of other peptides are affected.

This protein derivatization technique can be used to determine whether a candidate compound can be used, according to the invention to prevent productive HIV-1 infection

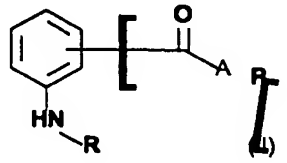
Treatment of Infectious Diseases

The compounds of formula I and formula II can be used to prevent or treat infectious diseases in animals, including mammals and preferably humans, and these compounds are particularly suited to treatment of parasitic diseases, particularly, malaria. The invention described herein provides methods for treatment of infection, including and without limitation, infection with parasites, and methods of preventing diseases associated with such infection. The compounds can reduce parasitemia when administered to an animal infected with a parasite.

Infectious diseases may include without limitation: protozoal diseases such as those caused by Kinetoplastida such as *Trypanosoma* and *Leishmania*, by Diplomonadina such as *Giardia*, by Trichomonadida such as *Dientamoeba* and *Trichomonas*, by Gymnamoebia such as *Naegleria* and the Amoebida such as *Entamoeba* and *Acanthamoeba*, by Sporozoasida such as *Babesia* and the Coccidiasina such as *Isospora, Toxoplasma, Cryptosporidium, Eimeria, Thelleria,* and *Plasmodium*; metazoal diseases such as those caused by the Nematoda (roundworms) such as *Ascaris, Toxocara,* the hookworms, *Strongyloides*, the

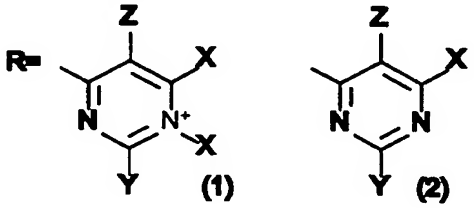
whipworms, the pinworms, *Dracunculus, Trichinella*, and the filarial worms, and by the Platyhelminthes (flatworms) such as the Trematoda such as *Schistosoma*, the blood flukes, liver flukes, intestinal flukes, and lung flukes, and the Cestoda such as the tapeworms; viral and chlamydial diseases including for instance those caused by the Poxviridae, Iridoviridae, Herpesviridae, Adenoviridae, Papovaviridae, Hepadnaviridae, Parvoviridae, Reoviridae, Birnaviridae, Togaviridae, Coronaviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Retroviridae, Picornaviridae, Calciviridae and by *Chlamydia*; bacterial diseases; mycobacterial diseases; spirochetal diseases; rickettsial diseases; and fungal diseases.

In one embodiment, the compounds of formula I and formula II having anti-infective activity are formed according to formula (I). In addition, the compounds of the invention, having anti-infective activity, can also be formed according to formula II:

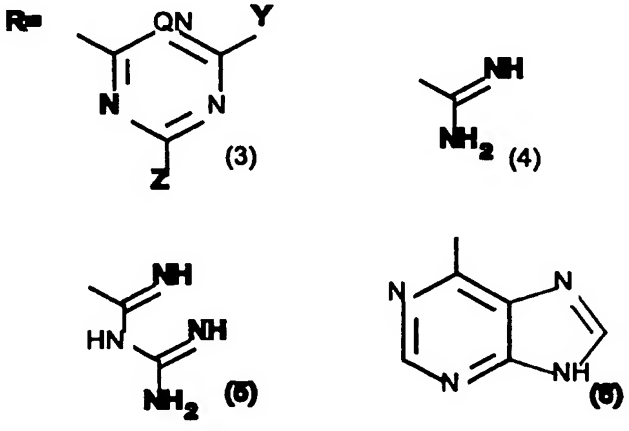


(I)

wherein A, independently, = CH_3 or CH_2CH_3 and P = 0, 1 or 2; and



wherein X = NH_2, CH_3 or CH_2CH_3; X' = CH_3 or CH_2CH_3; Y = NH_2, $NHCH_3$, $N(CH_3)_2$; and Z = H, CH_3 or CH_2CH_3; or



wherein Y' and Z', independently, = H, NH_2, $NHCH_3$, $N(CH_3)_2$ or $N^+(CH_3)_3$; Q is N or CH; and salts thereof.

In another embodiment, the compounds of the invention may be used therapeutically against infections with Plasmodium species, such as *P. falciparum, P. vivax, P. ovale and P. malariae*, that cause acute and recurrent malaria in humans. The compounds of the invention are also active against infection by other Plasmodium species, which include *P. berghei, P. knowlesi, P. simium, P. cynomolgi bastianelli* and *P. brasilianum.*

In yet another embodiment of the invention, the compounds may be useful in providing chemoprophylaxis for individuals at risk of infection, such as when traveling in endemic areas. By maintaining in circulation an effective concentration of a compound of the invention, malaria can be prevented by suppressing the pathological stages of infection with Plasmodium species. The compounds of formula I and formula II can be effective against various stages of the life cycle of the parasite, including sporozoites and merozoites, as well as dormant, asexual and sexual stages. The compounds of formula I and formula II may be active in the blood stream, in erythrocytes, in the liver, or in other tissues where the malaria parasite may reside.

In a specific embodiment, the compounds of formula I and formula II can be used to prevent malaria, or to treat malaria, or to treat infection with Plasmodium species that are resistant to antimalarial drugs, such as, but not limited to, chloroquine and pyrimethamine. The antimalarial properties of the compounds of formula I and formula II are not diminished against *P. falciparum* known to be resistant to chloroquine or pyrimethamine.

In a further embodiment, the compounds of formula I and formula II may contain a single acyl group (P=1) on the arylene ring or the acyl group can be absent therefrom (P=0) and/or the heterocyclic substituent (R can be uncharged). In the embodiment of the invention wherein there are two acyl groups (P=2) on the arylene ring, it is preferred that such acyl groups are not in an *ortho* arrangement relative to each other. The compounds that possess potent antimalarial activity are, preferably, arylene bis(methylketone) compounds that contain an uncharged heterocylic ring, such as a compound 11 (see Figure 4A).

The antimalarial properties can be analyzed by techniques, assays and experimental animal models, such as, the inhibition of growth of Plasmodium falciparum *in vitro* by a hypoxanthine-incorporation method (Desjardins et al., *Antimicrob. Ag. Chemother.* 16:710-718, 1979). The *in vitro* antiparasitic activities of several exemplary compounds of the invention were assessed by this method. The *in vivo* efficacy of the compounds can also be tested in mouse models in which parasitemia is enumerated following administration of a compound (Ager 1984, *Rodent malaria models*, pp 225-264. In *Handbook of Experimental Pharmacology* vol. 68, Antimalarial Drugs, Peters and Richards eds, Springer-Verlag, Berlin). The *in vivo* therapeutic activity of several exemplary compounds had been evaluated

in a four-day suppression model in mice.

In another embodiment, the invention provides a method of preventing or treating malaria by administering to a subject in need thereof an effective amount of a compounds of formula I and formula II. In a further aspect there is provided a method of preventing or treating malaria, especially malaria caused by drug resistant Plasmodium species in humans, which method comprise administering to the individual in need thereof an effective amount of a compounds of formula I and formula II and an effective amount of an antimalarial drug. The invention also provides the use of a compounds of formula I and formula II and an antimalarial drug in the manufacture of a medicament for the prevention or treatment of malaria. Such antimalarial drugs may include but are not limited to quinine, aminoquinolines (chloroquine and primaquine), pyrimethamine, mefloquine, halofantrine, and artemisinins.

The "adjunct administration" of a compound of the invention and an antimalarial drug means that the two are administered either as a mixture or sequentially. When administered sequentially, the compound may be administered before or after the antimalarial drug, so long as the first administered agent is still providing antimalarial activity in the animal when the second agent is administered. Any of the above-described modes of administration may be used in combination to deliver the compound and the antimalarial drug.

Example 1

This example illustrates the synthesis of several exemplary compounds. Compound 2 (Figure 1A) was made by suspending compound 11 (2-amino-4-(3,5-diacetylphenyl)amino-6-methylpyrimidine) (0.284 g) in 1:1 acetonitrile-tetrahydrofuran and treating with methyl iodide (2 mL) and heating at 40-45 °C under a reflux condenser for 18 hr. Cooling and filtration gave 0.35 g of 2-amino-4-(3,5-diacetylphenyl)amino-1,6-dimethylpyrimidinium iodide, mp 292 °C.

2-Amino-4-(3,5-diacetylphenyl)imino-1,4-dihydro-1,6-dimethylpyrimidine was made by suspending 21 g (49.3 mmole) of 2-amino-4-(3,5-diacetylphenyl)amino-1,6-dimethylpyrimidinium iodide (compound 2, synthesized above) in 1:1 methanol/water (750 mL) at 60 °C and treating with excess 2N NaOH with cooling to maintain about 60 °C. An additional 200 mL of water was added and the mixture was cooled in ice and filtered to give 14.69 g 2-amino-4-(3,5-diacetylphenyl)imino-1,4-dihydro-1,6-dimethylpyrimidine as yellow crystals, mp 219-220 °C.

2-Amino-4-(3,5-diacetylphenyl)amino-1,6-dimethylpyrimidinium chloride (CNI-0294). CNI-0294 is the chloride salt of compound 2. The base 2-amino-4-(3,5-diacetylphenyl)imino-1,4-dihydro-1,6-dimethylpyrimidine (14.35 g, 48 mmole) was dissolved in 500 mL of methanol and treated with HCl gas until precipitation appeared complete. Filtration gave 12.8 g of white crystals with a faint yellowish tinge, mp 306.5-307.5°.

Compound 11 (CNI-1194) was synthesized by suspending 3,5-diacetylaniline (0.885

g) in water (18 mL) and treating with 2-amino-4-chloro-6-methylpyrimidine (0.718 g) and concentrated HCl (0.42 mL) and heating at 90-100 °C for 30 min. After cooling the mixture was treated with 10 mL of aqueous 1N KOH. The mixture was stirred for 10 min and the solid was filtered out, washed with water, and dried, to give 1.332 g of tan crystals. Recrystallization from ethyl acetate-2-methoxyethanol gave 1.175 g of 2-amino-4-(3,5-diacetylphenyl)amino-6-methylpyrimidine as light buff crystals, mp 240-241°C.

Compound 12 was synthesized by suspending 3,5-diacetylaniline (0.531 g) in water (8 mL) and treating with cyanoguanidine (0.285 g) and conc. HCl (0.25 mL) and heating at reflux. After 6 hr the mixture was cooled and concentrated and 0.248 g of off-white solid was filtered out and dried to give N-(3,5-diacetylphenyl) biguanide hydrochloride, mp 260-70°C (dec).

Compound 13 was synthesized by suspending of 3,5-diacetylaniline (1.95 g) in water (10 mL) and treating with 2-chloro-4,6-diamino-1,3,5-triazine (1.455 g) and concentrated HCl (0.1 mL) and heating at reflux for 20 min. After cooling the hydrochloride of compound 13 separated as a white powder. This was filtered out, dissolved in 60 mL of boiling aqueous 75% methanol and treated with triethylamine (1.5 mL). On cooling, off-white flakes separated. Filtration and drying gave 1.79 g of 2-(3,5-diacetylphenyl)amino-4,6-diamino-1,3,5-triazine, mp 271-2°C.

Compound 14 4-(3-acetylphenyl)amino-2-amino-6-methylpyrimidine and compound 15 were synthesized (0.968 g) by suspending in acetone (5 mL) containing methyl iodide (2 mL) was heated at reflux for 48 hr. Filtration after cooling gave 0.657 g of 4-(3-acetylphenyl)amino-2-amino-1,6-dimethylpyrimidinium iodide as a white powder, mp 238-40 °C.

Compound 15 (CNI-1594) was synthesized by suspending m-aminoacetophenone (2.7 g) and 2-amino-4-chloro-6-methylpyrimidine (2.87 g) in 40 mL water and treating with 1.7 mL concentrated HCl and heated at reflux for 1 hour. Addition of 40 mL 1N KOH gave a light buff solid, which was filtered out and dried to give 3.8 g 4-(3-acetylphenyl)amino-2-amino-6-methylpyrimidine, mp 196-98 °C.

Compound 16 was synthesized by suspending 3,5-diacetylaniline (0.531 g) in water (10 mL) and treating with 6-chloropurine (0.464 g) and concentrated HCl (0.25 mL) and heating at reflux for 30 min. After cooling the mixture was treated with 6 mL of aqueous 1N KOH. The mixture was stirred for 10 min and the solid was filtered out, washed with water, and dried, to give 0.80 g of 6-[(3,5-diacetylphenyl)amino]purine, mp dec 340-350 °C.

Compound 17 (CNI-1794) was synthesized by suspending p-aminoacetophenone (1.35 g) and 2-amino-4-chloro-6-methyl pyrimidine (1.435 g) in 20 mL water and treating with 0.85 mL conc HCl and heating at reflux for 1 hr. Addition of 20 mL 1N KOH gave a light buff solid, which was filtered out and dried to give 2.28 g 4-(3-acetylphenyl)amino-2-amino-6-methylpyrimidine, mp 194-196 °C. Of this, 1.21 g was treated with methyl iodide (3 mL) in dimethylformamide (15 mL) at room temperature for 42 hr. Dilution with ethyl

acetate and filtration gave 1.11 g 4-(4-acetylphenyl)amino-2-amino-1.6-dimethyl pyrimidinium iodide as a white powder, mp 302-3°C.

Compound 45 (CNI-4594) was synthesized when a mixture of aniline (0.93 g) and 2-amino-4-chloro-6-methylpyrimidine (1.44 g) in 36 mL water was treated with 0.84 mL conc HCl and heated at reflux for 1 hr. Addition of 20 mL 1N KOH gave a light buff solid, which was filtered out, dried, and recrystallized from ethyl acetate/2-methoxyethanol and ethyl acetate/hexane to give 0.69 g 4-phenylamino-2-amino-6-methylpyrimidine, mp 179-180°C.

Compound 46 was synthesized by suspending 4-phenylamino-2-amino-6-methylpyrimidine, and compound 45, (0.25 g) in ethanol (4 mL) and treating with methyl methanesulfonate (0.090 g) and heating at reflux for 5 days. Additional methyl methanesulfonate (0.090 g) was added and the mixture refluxed another 2 days. Concentration and recrystallization from a mixture of methanol, ethyl acetate, and tert-butyl ethyl ether gave 0.10 g of 4-phenylamino-2-amino-1,6-dimethylpyrimidinium methanesulfonate.

3,5-diacetylaniline (CNI-1894) was synthesized as described in Ulrich et al. (*J. Med. Chem.* 27:35-40, 1983). Diacetylanilines substituted in other positions was synthesized according to Ulrich et al. *supra* or McKinnon et al. (*Can. J. Chem.* 49:2019-2022, 1971). All other starting materials were obtained from the Aldrich Chemical Co. Nuclear magnetic resonance spectra and elemental analysis for all the compounds agreed with expected values.

Example 2

This example illustrates the use of a compound to inhibit HIV replication in primary macrophage lines. Primary human monocytes were obtained from peripheral blood by Ficoll-Hypaque centrifugation and adherence to plastic (Gartner et al., *Science* 233:215, 1986). Briefly, after Ficoll-Hypaque (Pharmacia) separation, PBMCs were washed 4 times with DMEM (the last wash was done at 800 rpm to remove platelets) and resuspended in monocyte culture medium [DMEM supplemented with 1 mM glutamine, 10% heat-inactivated human serum, 1% penicillin+streptomycin mixture (Sigma)] at a density of $6x10^6$ cells/ml. Cells were seeded in 24-well plates (1 ml per well) and incubated for 2 h at 37 °C, 5% CO_2. Following incubation, cells were washed 3 times with DMEM to remove non-adherent cells and incubation was continued in monocyte culture medium supplemented with 250 U/ml human M-CSF (Sigma). Cells were allowed to mature for 7 days prior to infection with the monocyte-tropic strain, HIV-1_{ADA} (Nuovo et al., *Diagn. Mol. Pathol.* 1:98, 1992). Two hours after infection, cells were washed with medium and cultured in RPMI supplemented with 10% human serum. In experiments where PCR analysis was performed, virus was pretreated with RNAse-free DNAse (Boehinger-Mannheim) for 2 h at room temperature and then filtered though a 0.2 μm pore nitrocellulose filter prior to infection.

PBMCs were purified by Ficoll-Hypaque centrifugation and activated by 10 μg/ml PHA-P (Sigma) and 20 U/ml recombinant human IL-2 (rhIL-2) in RPMI 1640 supplemented

with 10% FBS (HyClone). After 24 h incubation, cells were washed and inoculated with HIV-1_{ADA} in RPMI 1640 supplemented with 10% FBS. After a 2 h adsorption, free virus was washed away and cells were cultured in RPMI 1640 supplemented with 10% FBS and 20 U/ml rhIL-2.

Macrophage-tropic strain HIV-1_{ADA} was amplified in primary human monocytes and concentrated to produce stock with $TCID_{50}$ of about 10^5/ml. The concentration of HIV-1 was determined by immunoassay of viral p24, concentration; using a conversion factor of 1 ng / 200 HIV-1 particles.

For p24 assay, sequential 1:9 dilutions of culture supernatant were prepared and analyzed by ELISA as suggested by the manufacturer (Cellular Products, Buffalo, NY). For the reverse transcriptase (RT) assay, 10 µl of culture supernatant was added to 40 µl of reaction mixture (final composition was 50 mM Tris-HCl, pH 7.8; 20 mM KCl; 5 mM $MgCl_2$; 1 mM DTT; 0.1% Triton X-100; 0.2 OD/ml polyA; 0.2 OD/ml oligo$(dT)_{12-18}$; and 40 µCi/ml ^{3}H-dTTP (76 Ci/mmol, DuPont) and incubated 2 hr at 37°C. 5 µl of the reaction mixture was then spotted onto the DE 81 (Whatman) paper. Paper was air dried and washed 5 times with 5% Na_2HPO_4, followed by rinsing with distilled water. After air drying, paper was put on a Flexi Filter plate (Packard), covered with scintillation fluid and counted in a Top Count Microplate Counter (Packard). Results are expressed as counts per minute in 1 ml of supernatant (cpm/ml).

The cytotoxicity of compound 2 was tested in monocyte cultures by trypan blue exclusion assay or lactate dehydrogenase (LDH) release. By both assays, no cytotoxic effect was observed with concentrations of the compound up to 10 mM (data not shown). Results presented in Figure 2 show the effect of various concentrations of compound 2 on HIV-1 replication in monocytes. The IC_{50} for this compound was between 0.1 and 1 nM. Similar and higher concentrations of the compound were also tested on activated PBLs. The anti-viral effect of this compound was much less expressed in these actively dividing cell populations (Figure 3). No anti-viral effect was detected when cultures of replicating cells were infected at the multiplicity of infection used to infect monocytes.

AZT is a drug that is routinely used to treat HIV-1 infected persons. However, two factors are known to diminish the effectiveness of AZT: its toxicity and the emergence of resistant mutant strains of HIV-1. The effects of both of these factors can be reduced by administering a second, synergistic HIV-1-inhibitory drug with AZT.

In view of these premises, the effects on HIV-1 replication in human monocyte cultures of the various concentrations of AZT, alone or in combination with 100 nM compound 2, were tested using the protocols described above. Drugs were added to the monocyte cultures together with HIV-1 at about 10^5 TCID / ml. The concentration of drugs was maintained on refeeding. HIV-1 replication was assessed by assay of the supernatant for reverse transcriptase activity. The results are expressed as mean ± std. dev. ($cpmx10^{-3}$) in Table 3.

TABLE 3

Effects of Combined AZT/compound 2 on HIV-1 infected Monocyte Cultures

	day-7		day-11	
[AZT]	(-) No. 2	(+) No. 2	(-) No. 2	(+) No. 2
0	1.46 ± 0.43	0.37 ± 0.07	1.81 ± 0.75	0.72 ± 0.30
10 pM	0.92 ± 0.21	0.15 ± 0.05	1.63 ± 0.81	0.18 ± 0.06
100 pM	0.79 ± 0.14	0.13 ± 0.04	1.34 ± 0.59	0.15 ± 0.06
1 nM	0.60 ± 0.28	0.04 ± 0.02	1.07 ± 0.49	0.09 ± 0.03
10 nM	0.05 ± 0.02	0.03 ± 0.02	0.08 ± 0.03	0.07 ± 0.03

These results demonstrate that there is synergy between reverse transcriptase inhibitors (exemplified by AZT) and compound that act to inhibit nuclear importation of the HIV preintegration complex (exemplified by compound 2). The synergistic effects are most pronounced at the lower doses of AZT on day 11. For example, 10 pM AZT alone produces an about 20% reduction in RT activity on day-11, 100 nM compound 2 alone produces about a 60% reduction. Without synergy, the combination should produce a 70% reduction (100 x (1- (.8 x.4)). Instead the observed reduction was 90%.

Example 3

This example illustrates that compound 2 directly inhibits nuclear importation of HIV-1 preintegration complexes and this can be directly measured by detecting the presence of circularized duplex HIV-1 genomic DNA. These duplex circles can be readily detected by PCR amplification using primers which span the junction of the circularized HIV-1 genome Bukrinsky et al., *Proc. Natl. Acad. Sci. USA* 89:6580-84, 1992).

Briefly, the efficiency of nuclear translocation was estimated by the ratio between the 2-LTR- and *pol* -specific PCR products, which reflected the portion of 2-LTR circle DNA molecules as a fraction of the entire pool of intracellular HIV-1' DNA. Viral 2-LTR circle DNA is formed exclusively within the nucleus of infected cells and thus is a convenient marker of successful nuclear translocation (Bukrinsky, *Nature* 365:666-669, 1993).

Total DNA was extracted from HIV-1-infected cells using an IsoQuick extraction kit (Microprobe Corp., Garden Grove, CA). DNA was then analyzed by PCR using primer pairs that amplify the following sequences: a fragment of HIV-1 (LTR/gag) that is the last one to be synthesized during reverse transcription and therefore represents the pool of full-length viral DNA molecules; a fragment of polymerase gene (*pol*); a 2-LTR junction region found only in HIV-1 2-LTR circle DNA molecules; or a fragment of the cellular a-tubulin gene. Dilutions of 8E5 cells (containing 1 integrated copy of HIV-1 DNA per genome) into CEM

cells were used as standards. Amplification products were transferred to nylon membrane filters and hybridized to ^{32}P-labeled oligonucleotides corresponding to internal sequences specific for each PCR amplification fragment, followed by exposure to Kodak XAR-5 film or a phosphor screen.

Bands of correct size revealed after hybridization were quantitated with a PhosphorImager (Molecular Dynamics) by measuring the total density (integrated volume) of rectangles enclosing the corresponding product band. Efficiency of nuclear translocation of HIV-1 DNA was estimated by measurement of the amount of 2-LTR circle DNA ($N_{2\text{-}LTR}$) relative to total viral DNA (N_{tot}) in each culture, indexed to the same ratio of appropriate control cultures. Thus, the Translocation Index = $(N_{2\text{-}LTR}/N_{tot}) / (C_{2\text{-}LTR}/C_{tot}) \times 100$.

Primary human monocytes were infected with HIV-1_{ADA} in the presence of 100 nM concentration of compound 2 or without drug (control). Half the medium was changed every 3 days, and drug was present throughout the whole experiment. Cell samples were taken at 48 and 96 hours post infection and the Translocation Index, relative to the drug free control was determined. At both time points the Translocation Index was less than 10, indicating there was greater than 90% inhibition of nuclear importation.

Example 4

This example illustrates the toxicological and pharmacokinetic properties of exemplary compounds. Standard addition curves for each test compound were constructed by adding increased amounts of drug to mouse or human A^+ plasma (Long Island Blood Services; Melville, NY). An equal volume of 10 μM tetramethylammonium chloride/10 μM heptane sulfonate/4.2 mM H_3PO_4 (Buffer A) was added to the plasma sample, which was then loaded onto a washed 1 g cyanopropylsilane (or octadecylsilane for CNI-1894) solid-phase extraction column (Fisher Scientific). The column was washed with 1.0 ml of water and then eluted with 1.0 ml of 10 μM tetramethylammonium chloride/10 μM heptane sulfonate/4.2 mM H_3PO_4/95% CH_3CN/5% H_2O (Buffer C). The eluted sample was reduced to dryness in a rotary evaporator and resuspended in 1.0 ml Buffer A.

Two hundred ml of the resuspended sample was injected onto a Hewlett-Packard model 1090 high performance liquid chromatography system (HPLC)(Wilmington, DE) equipped with a photodiode array ultraviolet/visible spectrophotometric detector, autosampler, and Chemstation operating software. The column used was a 250 x 4.6 mm Zorbax RX-C8 column (Mac-Mod Analyticals; Chadd's Ford, PA) kept at room temperature and run at 1.5 ml/min. The mobile phase used was Buffer A and 10 μM tetramethylammonium chloride/10 μM heptane sulfonate/4.2 mM H_3PO_4/75% CH_3CN/25% H_2O (Buffer B), with all runs initiated at 10% Buffer B. A linear 30 min gradient to 60% Buffer B was then performed, followed by a 4 min reverse gradient to initial conditions. Compounds CNI-0294, -1194, -1594, and -1794 were detected by ultraviolet absorbance at 300 nm, CNI-1894 at 240 nm, and pentamidine at 265 nm. In this assay system, the CNI test

compounds have a linear response and are detectable down to at least 19.5 ng per injection.

The doses of compounds of the invention found to be lethal to 50% of the mice (LD_{50}) were determined by intraperitoneal injection of groups of five animals with increasing doses of each compound. CNI-0294 was administered from 0, 2, 10, 20, 40, 80, 160, 320, 640, 1280 mg/kg in 0.5 ml of water/HCl; CNI-1594 at 0, 2.4, 5, 10, 20, 40, 80 mg/kg in 0.5 ml of water/HCl; CNI-1794 at 0, 20, 50, 80 mg/kg in 0.5 ml of water/HCl; and CNI-1894 at 0, 10, 20, 40, 80, 240, 480, 960 mg/kg in water/HCl. All animals were observed for visible signs of acute or long-term toxicity. The percentage of animals in each group which died were utilized to calculate the LD_{50} by non-linear curve fitting with the Enzfit software (Elsevier Bioscience; Cambridge, UK) programmed with the Chou equation (Chou, *J. Theor. Biol.* 39:253-276, 1976).

The compounds (Figure 4A-E), were screened for toxicity via a modified LD_{50} assay procedure as described above in an outbred strain of mice. The results are shown in Table 4 as follows:

Table 4 The toxicity of the CNI compounds, as measured by the median lethal dose determined as described above.

Compound	LD_{50}±standard deviation (mg/kg)
0294	587.77±65.79
1194	>160*
1594	49.04±0.08
1794	48.93±0.12
1894	258.64±1.37

Higher doses were not tested due to limiting amounts of the compound. CNI-0294 (compound 2) was found to be very well tolerated (see Table 4), with no overt signs of toxicity detectable at doses approaching the LD_{50}. The other compounds in the CNI series were designed to allow for structure-function relationships with respect to activity and toxicity. CNI-1194, which differs from CNI-0294 only by the lack of a methyl group on the heterocyclic nitrogen, was also well tolerated, with a high LD_{50} (Table 4). However, CNI-1594, which is similar to CNI-1194 plus the omission of one of the acetyl groups on the benzene rings, was much more lethal (Table 4). This toxicity was immediate, with death occurring in minutes and the animals displaying signs of acute neurotoxicity. CNI-1794, which is identical to CNI-1594 except that the single acetyl group is moved para to the heterocyclic substituent, had an LD_{50} identical to that for CNI-1594 (Table 4). CNI-1894, which is similar to CNI-0294 and -1194 but lacks the heterocyclic ring, was also reasonably well tolerated. Animals dosed with large amounts of CNI-1894 died 2-3 days post injection, and showed no sign of any immediate toxicity. Based on the above observation, it is concluded that the presence of the heterocyclic ring in the compounds plays only a small role in determining toxicity, while the presence of two acetyl groups on the benzene ring is very

important. Therefore, a preferred compound, showing low toxicity, contains two acetyl groups on the benzene ring.

Female ND4 Swiss Webster mice (21-24 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and randomly placed in groups of five in cages with free access to food and water. Each group of animals received 50 mg/kg of CNI-0294, -1194, or -1894, or 20 mg/kg of CNI-1594 in a volume of 0.5 ml. Compound CNI-0294 was administered intraperitoneally or by oral gavage as a solution in water or a suspension in 10% DMSO/peanut oil. The other compounds were administered intraperitoneally or by oral gavage as a solution in water titrated with sufficient HCl to dissolve the drug. At various time points, ranging from 5 min to 4 days, a single group of animals was euthanized by carbon dioxide inhalation and bled by cardiac puncture using heparin as an anticoagulant. The blood from the five mice in the group was pooled and centrifuged at 14000 x g for 10 min. The volume of plasma was measured, and equal volume of Buffer A added, and the mixture extracted and analyzed as described above, except that the dried eluates were resuspended in 200 ml Buffer A and 100 ml was injected onto the high performance liquid chromatography (HPLC) system.

As inspection of the blood concentration-time curves for a single i.p. injection showed a typical biphasic appearance, standard methods of pharmacokinetic measurement were employed (Gibaldi et al., *Pharmacokinetics* Marcel Dekker, New York, 1982). The area under the plasma concentration-time curve (AUC) was determined, and bioavailability was measured as $AUC_{oral}/AUC_{i.p.}$ A and B represent the zero time intercept of the distribution and elimination phases respectively, and a and b the respective slopes of the phases multiplied by 2.303. The $t_{1/2a}$ and $t_{1/2b}$ are calculated half-lives of the drug in each phase (0.693/a and 0.693/b respectively). The volume of distribution (V_D) was calculated as dose/B, and the total clearance rate (Cl_{tot}) calculated as b^*V_D. C_{max} and t_{max} are the maximal plasma concentration and time of this measurement, respectively.

As judged by the plasma concentration-time curves from a single intraperitoneal injection, each compound in the series had similar pharmacokinetic properties despite the structural differences. The kinetic parameters are summarized in Table 2 and a typical pattern is shown for CNI-1194 in Figure 5. The drugs were rapidly absorbed, with the maximal plasma concentration reached in 5-15 min, and also had a rapid distribution phase, with a $t_{1/2a}$ of 0.32-0.62 hr. Differences were found to occur in the maximal plasma concentration and parameters related to the elimination phase. CNI-0294 achieved the highest maximal plasma level for a single 50 mg/kg i.p. injection, with 18.76 mg/ml, and CNI-1894 was very similar with a value of 13.43 mg/ml. As CNI-1194 had an appreciably lower maximal plasma level and a slower t_{max} when compared with CNI-0294, it appears that the presence of the methyl substituent on the heterocyclic nitrogen enhances drug absorption from the peritoneum. A comparison of CNI-1194 and CNI-1594 implied that the number of acetyl groups had little effect on drug absorption. The values relating to elimination (b, B, $t_{1/2b}$, V_d, Cl_{tot}) were found

to vary, but no clear structural relationship could be discerned. All the compounds, except CNI-1894, were undetectable in plasma after 24 hr and approached the limit of detection after 5-6 hr. Therefore, as a general property, the compounds are absorbed and eliminated rapidly.

Experiments were also performed with CNI-0294 and -1194 to evaluate relative bioavailability. By comparing the AUC_{oral} against the $AUC_{i.p.}$ for a single 50 mg/kg dose, CNI-0294 was found to have 6% relative bioavailability and CNI-1194 15%. The maximal plasma level was 0.4 mg/ml for CNI-0294 and 0.35 mg/ml for CNI-1194, and the drugs were detectable in plasma for at least 6 hr (see Figure 5).

In a preliminary pharmacologic screening assay with compounds 2 and 11 for binding to 50 known receptors (including adenosines, adrenergics, calcium channels, estrogen, GABA, glutamates, histimine, muscarinic, opiate, potassium channel, serotonin, testosterone, sodium channel), the only binding activity was found with the muscarinic M_2 and M_3 receptors, in a dose-response fashion. These data indicate that the exemplary compounds may have some slight cholinergic activity.

During the analysis of the plasma samples for the pharmacokinetic parameters, a number of additional HPLC peaks were detected which increased and decreased over time. Extra peaks of this nature were seen in samples from each of the CNI series, as shown in Figures 8A-8D. As it was possible that these peaks represented metabolites of the CNI compounds, the compounds of the invention were screened in a simple model of primary metabolism.

Several female ND4 Swiss Webster mice were euthanized by carbon dioxide inhalation and the livers excised and rinsed with ice cold phosphate buffered saline (pH 7.4). The livers were minced, gently homogenized in 50 mM phosphate buffer (pH 7.4) with a Dounce homogenizer, and centrifuged at 9600 x g for 20 min. The post-mitochondrial supernatant was kept, glycerol added to 20%, and frozen at -70 °C in 1.0 ml aliquots until used. For each incubation, 1.0 ml of a 1.0 mg/ml drug solution was added to 3.0 ml of 50 mM phosphate buffer (pH 7.4), 1.0 ml of 2 mg/ml NADPH in 50 mM phosphate (pH 7.4), and 1.0 ml of the post-mitochondrial supernatant. Five hundred ml of each incubate was then immediately transferred to an ice-cold tube to provide the zero-time sample, and addition 500 ml aliquots removed to ice-cold tubes at 8, 15, 30, and 60 min. The samples were then extracted, and analyzed by HPLC. Control incubations were also performed where drug or post-mitochondrial supernatant was omitted. An incubation using pentamidine was performed to confirm microsomal activity (Berger et al., *Antimicrob. Ag. Chemother.* 36:1825-1831, 1992). Peaks in the compound incubations, which increased over time, and were not present in control samples lacking the enzyme preparation were treated as putative metabolites.

Using post-mitochondrial supernatants of homogenized mouse livers as a source of enzyme, the drugs were incubated in the presence of NADPH. As described in Berger et al. *supra*, pentamidine was used as a positive control, and the seven, expected, primary

metabolites were detectable, confirming the activity of the enzyme preparation. Extraction and analysis of the CNI incubates showed the presence of numerous, putative metabolite peaks that were not present in negative control incubations (Figure 6). Incubation of CNI-0294, -1594, or -1194 was found to produce three minor and one major metabolite and CNI-1894 had one minor and one major metabolite. The major metabolite was found to elute 0.9-1.2 min closer to the solvent front for CNI-0294, -1194, and -1594, suggesting that the same position was being altered in each of these compounds. The metabolic conversion in the post-mitochondrial supernatant system was considerable, with 43.5% of CNI-0294, 65.19% of CNI-1194, 11.74% of CNI-1594, and 17.28% of CNI-1894 altered during the course of a 60 min incubation (as judged by peak area). These results indicated that appreciable metabolism of the compounds of the invention should occur *in vivo*.

Re-examination of the plasma samples confirmed that the several of the unknown plasma peaks seen in Figures 6A and 6B corresponded to the putative metabolites in Figures 7A-7D. However, the metabolic model system did not produce all the unknown peaks seen in the plasma samples. In particular, a plasma peak eluting at 11-14 min was seen with all the compounds *in vivo*, but not seen at all in the *in vitro* test system. As was evident from the plasma time-course samples, there appeared to be a large amount of metabolic conversion *in vivo* of all of the compounds, regardless of the route of administration.

The toxicity, pharmacokinetics, and metabolism of the arylene bis(methylketone) compounds, and several analogues thereof were examined in mice. With a median lethal dose of 587.77 mg/kg, CNI-0294 was well tolerated when administered intraperitoneally. Analogues which also had two acetyl groups on the phenyl moiety were also well tolerated, with median lethal doses exceeding 160 mg/kg i.p. All visible toxic reactions appeared to be rather delayed (generally 2-3 days post injection). While no biopsy samples were taken, such a delay would be consistent with organ damage by very high doses these compounds. Compounds which had only one acetyl group were found to be more toxic, with median lethal doses of 48.93-49.04 mg/kg i.p. While the visible symptoms following injection of CNI-1594 or -1794 suggested a lethal neurotoxicity, the structural differences between the two drugs indicate that antagonism of an endogenous neurotransmitter is unlikely.

In test animals, all of the compounds possessed very rapid pharmacokinetic properties, with the plasma maximal concentration, for intraperitoneal injection, being reached in 5-15 min, and 15-60 min for oral dosing. For CNI-0294, a plasma maximal concentration of 18.76-18.93 mg/ml was reached after injection of 50 mg/kg i.p. The other compounds tested achieved lower maximal plasma levels (1.9-13.43 mg/ml). The half-life of the distribution phase ($t_{1/2a}$) was 0.32-0.62 hours, and that for the elimination phase ($t_{1/2b}$) was 3.65-23.10 hours. All of the kinetic parameters are consistent with drugs that are very rapidly cleared from the plasma and are not retained in tissues for a long period of time. Both CNI-0294 and -1194 were orally absorbed, with a relative bioavailability of 6 and 15 percent respectively. This latter feature is very favorable for continued development of these

compounds as anti-infective agents, particularly as antiviral and antiparasitic agents, and more particularly as anti-retroviral and anti-protozoal agents, and yet particularly as anti-HIV agents and antimalarials. The toxicity, kinetic, and bioavailability data suggest that frequent, high, oral doses of the CNI-0294 can safely maintain therapeutically effective plasma concentration.

Metabolism of the drugs was assessed in a mouse liver post-mitochondrial supernatant system, and extensive metabolism was discovered (11.74-65.19% metabolized during, a 60 minute incubation). Examination of plasma samples showed that there was considerable *in vivo* metabolism, with at least 4-6 metabolites easily detected during the first 3 hours following i.p. administration of the test compounds. The levels of metabolite rapidly exceeded plasma concentrations of the parent compound. The HPLC retention times indicated that the compounds were likely altered in the same positions. In addition, the metabolites, like the parent compounds, appeared to have very rapid plasma kinetics.

Example 5

This example illustrates anti-malarial therapeutic activity of several exemplary compounds. The antimalarial activity of the compounds was determined essentially as described in Desjardins et al. *supra*. Fifty ml of various concentrations of a compound, chloroquine, or pyrimethamine was added to the wells of microtiter plates, followed by 200 ml of ring-stage, synchronized, *P. falciparum*-infected erythrocytes (final hematocrit = 1.5%, final parasitemia = 1-5%). The plates were incubated for 24 hr in a candle jar kept at 37 °C, and then 25 ml of [^{3}H]-hypoxanthine (Amersham, Arlington Heights, IL; 2.5 µl Ci/well) was added. The plates were then incubated for a further 24 hr, before harvesting onto Unifilter-96 GF/C filter-microplates (Packard; Meriden, CT). Twenty-five ml of Microscint scintillation fluid (Packard) was added to each well of the filter-microplate, which was subsequently counted in a Top-count microplate scintillation counter (Packard). The percent of [^{3}H]-hypoxanthine uptake relative to control infect-erythrocytes was used to determine the IC_{50} value for the compounds by non-linear regression for LD_{50} determination.

Using the hypoxanthine-incorporation method for assessing *Plasmodium falciparum* growth *in vitro* as described above, CNI-0294 was found to have considerable anti-malarial activity (Table 5).

Table 5 The antimalarial activity of CNI-0294, chloroquine, and pyrimethamine *in vitro* against several *Plasmodium falciparum* clones. The median inhibitory concentration was determined as described above.

Clone	Chloroquine IC_{50}	Pyrimethamine IC_{50} (mM)	CNI-0294 IC_{50} (mM)
D10	26.99±2.42*	170.70±24.60	4.00±0.41
Dd2	122.54±7.26	103.70±9.79	3.52±0.10
FCR-3	104.68±9.98	0.04±0.01	3.09±0.30

HB3	6.73±0.16	8.97±2.75	1.79±0.27
W2mcf	143.79±13.30	17.81±13.46	2.29±0.22

* Each value is ± standard deviation (n=4 for chloroquine and CNI-0294, and n=2 for pyrimethamine).

The median inhibitory concentration (IC_{50}) for CNI-0294 was calculated to be 1.79-4.00 mM for a series of cloned parasites which have different sensitivities to chloroquine or pyrimethamine (Table 5).

The Dd2 clone of *P.falciparum*, which was both chloroquine and pyrimethamine resistant, was utilized to compare the antimalarial activity of the remaining CNI compounds (Table 6).

Table 6 The antimalarial activities of the compounds against the chloroquine- and pyrimethamine-resistant *P. falciparum* clone Dd2. The median inhibitory concentration was determined as described above.

Compound	IC_{50}±standard deviation (mM)
0294	3.67±0.57*
1194	20.27±1.62
1594	23.73±0.59
1894	>200**
4594	25.11±0.72

* n=4 for all. The CNI-0294 replicates were independent of those shown in Table 5.

** Highest concentration tested.

In independent measurements, CNI-0294 agreed well with the results in Table 5, and CNI-1194 was found to be approximately 5 fold less active. This difference suggested that the heterocyclic methyl group is required for maximal activity. CNI-1594 had an IC_{50} equal to that for CNI-1194 or CNI-4594 demonstrating that loss of one or both of the acetyl groups can have little effect on the antimalarial activity. CNI-1894, however, was inactive at the highest concentration tested.

The antimalarial activity of CNI-0294 *in vivo* was assessed by infecting female ND4 Swiss Webster mice with 100 µl of *Plasmodium berghei* NYU-2 infected mouse erythrocytes (50% parasitemia) by intraperitoneal injection. The animals were subsequently injected intraperitoneally once per day on days 1-4 of the infection with 0.5 ml water or 0.5 ml of 50 mg/kg CNI-0294 in water. Four hours after the final injection, small blood samples were taken from the tail, and thin smears stained with Dif-Quick (Baxter, Miami, FL). The parasitemia of control and treated animals was enumerated by inspection of at least 1000 erythrocytes in each animal.

As the CNI-0294 IC_{50} for *P.falciparum* was in the range achieved for approximately one hr following a single i.p. injection of 50 mg/kg in mice, the compound was also screened *in vivo* in mice infected with *Plasmodium berghei*. Utilizing the four day suppression test, where parasitemia is enumerated following four daily injections of the test compound (in this

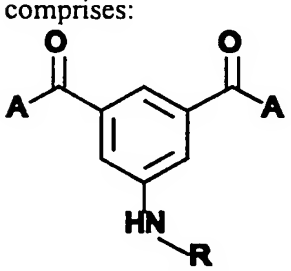
case 50 mg/kg i.p.), CNI-0294 was found to significantly ($P \leq 0.01$) lower the parasitemia by 10-fold (Figure 9).

As indicated in Table 5, CNI-0294 was effective against various clones of *P. falciparum*. The consistency in CNI-0294 IC_{50} over such a range of chloroquine and pyrimethamine IC_{50}'s suggested that CNI-0294 had a different mechanism of action than either of these established antimalarials.

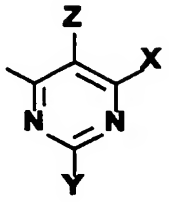
While daily 50 mg/kg injections i.p., for 4 days, were found to strongly suppress *P. berghei* infection in mice, these animals were not completely cured during this course of treatment. The difference between these *in vivo* results and the more striking *P. falciparum in vitro* results are likely due to the kinetic and metabolic properties of the compound. *In vitro*, the parasites are exposed to a constant level of the drug for 48 hr, with no source of host metabolizing enzymes. In the case *in vivo*, the single, daily i.p. injection only provides therapeutic plasma concentrations for approximately one hour and there is considerable metabolism to compounds which may have reduced anti-plasmodial activity. In light of these observations, one of ordinary skill in the art would be able to further optimize the dosing regimens.

WE CLAIM:

1. A pharmaceutical composition for oral administration, comprising a compound of formula I in a buffered oral pharmaceutical formulation, wherein formula I comprises:



wherein A = CH_3 or CH_2CH_3 and R is

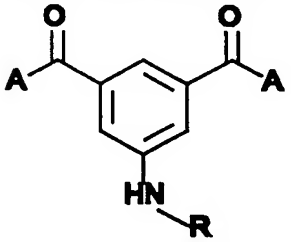


wherein X = NH_2, CH_3 or CH_2CH_3; Y = NH_2, $NHCH_3$, $N(CH_3)_2$; and Z = H, CH_3 or CH_2CH_3.

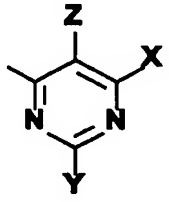
2. A method for preventing productive infection by a virus in a terminally differentiated cell having a nucleus, which comprises the step of preventing importation of a particle containing a viral genome into the nucleus.

3. The method of claim 2 which comprises administering an effective amount of a pharmaceutical composition containing an arylene bis (methyl carbonyl) as an active ingredient.

4. The method of claim 3 wherein the pharmaceutical composition contains a compound from formula I, wherein formula I comprises:



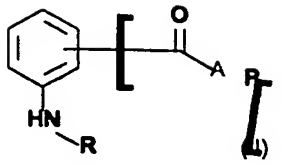
wherein A = CH_3 or CH_2CH_3 and R is



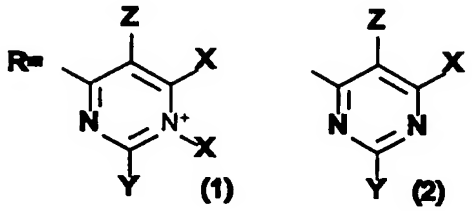
wherein X = NH_2, CH_3 or CH_2CH_3; Y = NH_2, $NHCH_3$, $N(CH_3)_2$; and Z = H, CH_3 or CH_2CH_3.

5. The method of claim 2 wherein the virus is HIV-1.

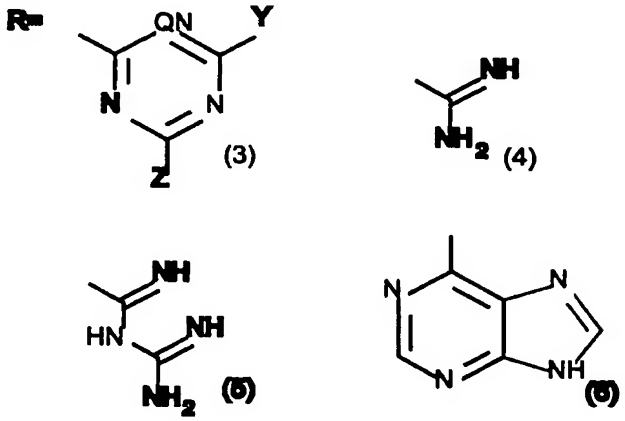
6. A method for treating or preventing an infectious disease in an animal comprising administering to the animal an effective amount of a compound according to the formula:



wherein A, independently, = CH_3 or CH_2CH_3; P = 0, 1 or 2; and



wherein X = NH_2, CH_3 or CH_2CH_3; X' = CH_3 or CH_2CH_3; Y = NH_2, $NHCH_3$, $N(CH_3)_2$; and Z = H, CH_3 or CH_2CH_3; or



wherein Y' and Z', independently, = H, NH_2, $NHCH_3$, $N(CH_3)_2$ or $N^+(CH_3)_3$; Q is N or CH; and salts thereof.

7. The method of claim 6 wherein the compound is an arylene bis (methyl ketone).

8. The method of claim 8 wherein the compound is compound 11.

9. The method of claim 6 wherein the infectious disease is malaria.

10. The method of claim 9 wherein the malaria is caused by *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* or *Plasmodium malariae*.

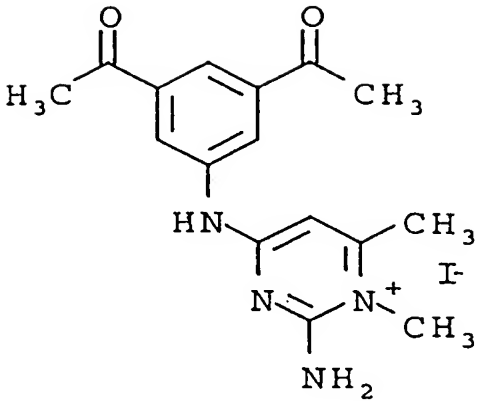


FIG. 1A

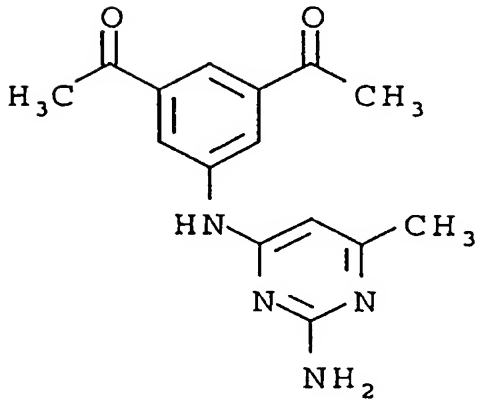


FIG. 1B

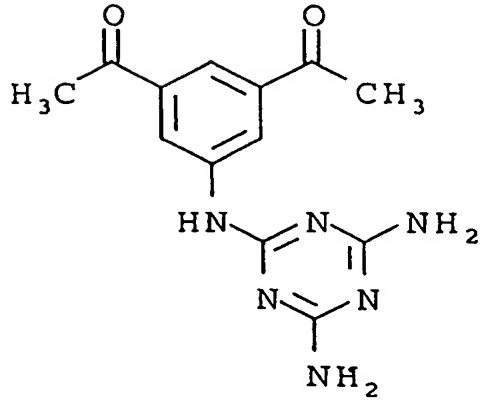


FIG. 1C

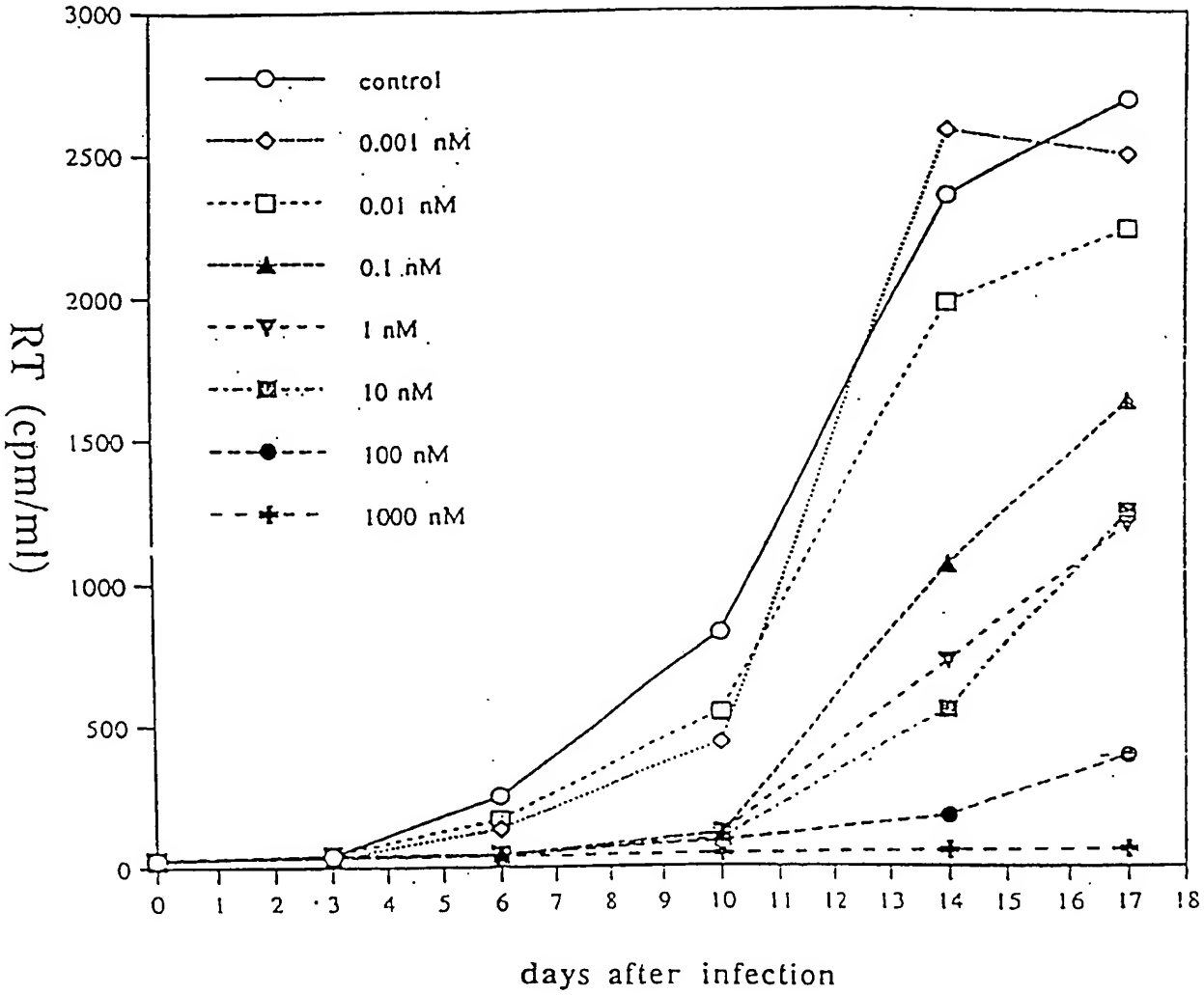

3000
2500
2000
1500
1000
500
0
RT (cpm/ml)
control
0.001 nM
0.01 nM
0.1 nM
1 nM
10 nM
100 nM
1000 nM
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
days after infection

FIG. 2A

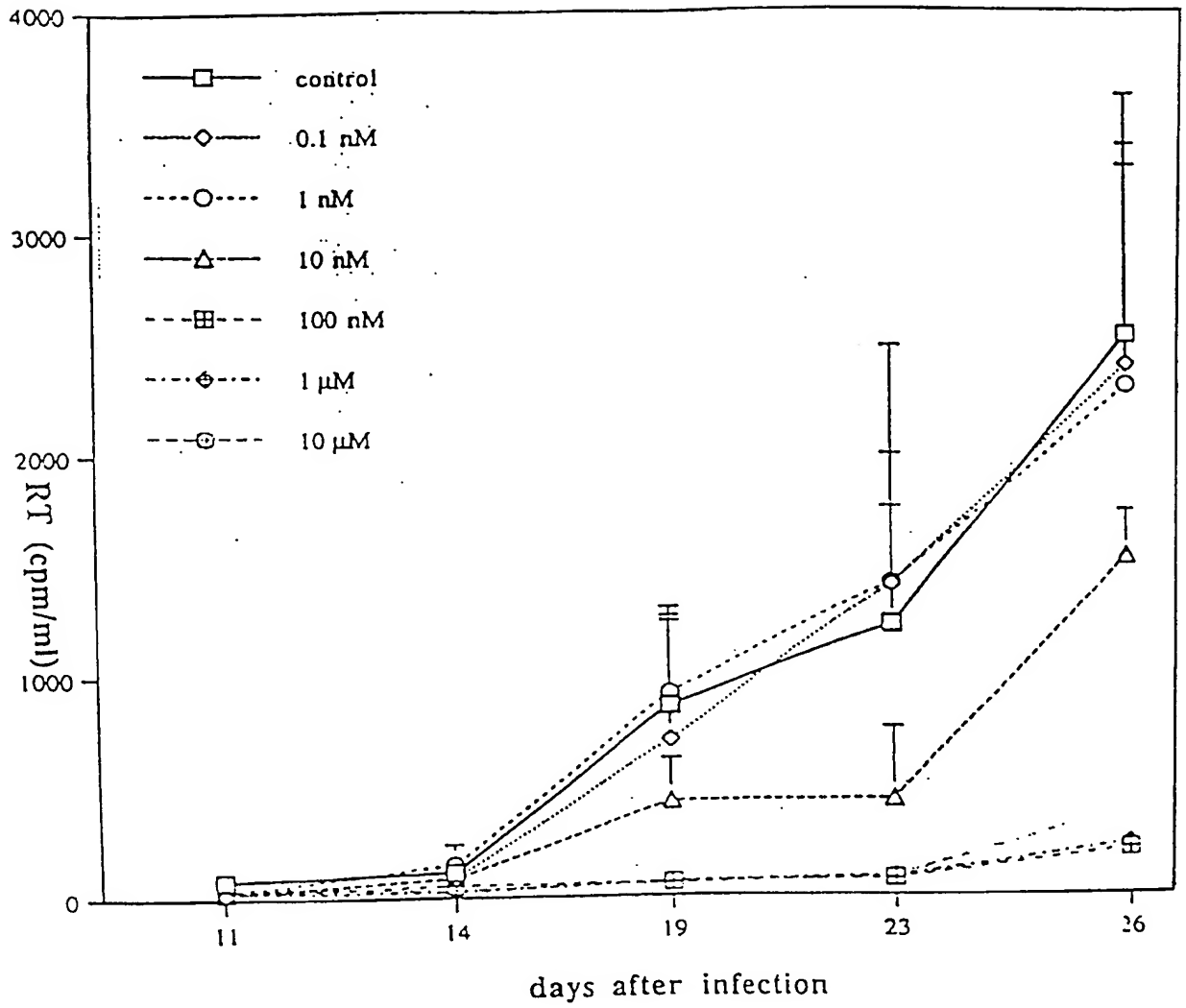

4000
3000
2000
1000
0
RT (cpm/ml)
control
0.1 nM
1 nM
10 nM
100 nM
1 µM
10 µM
11
14
19
23
26
days after infection

FIG. 2B

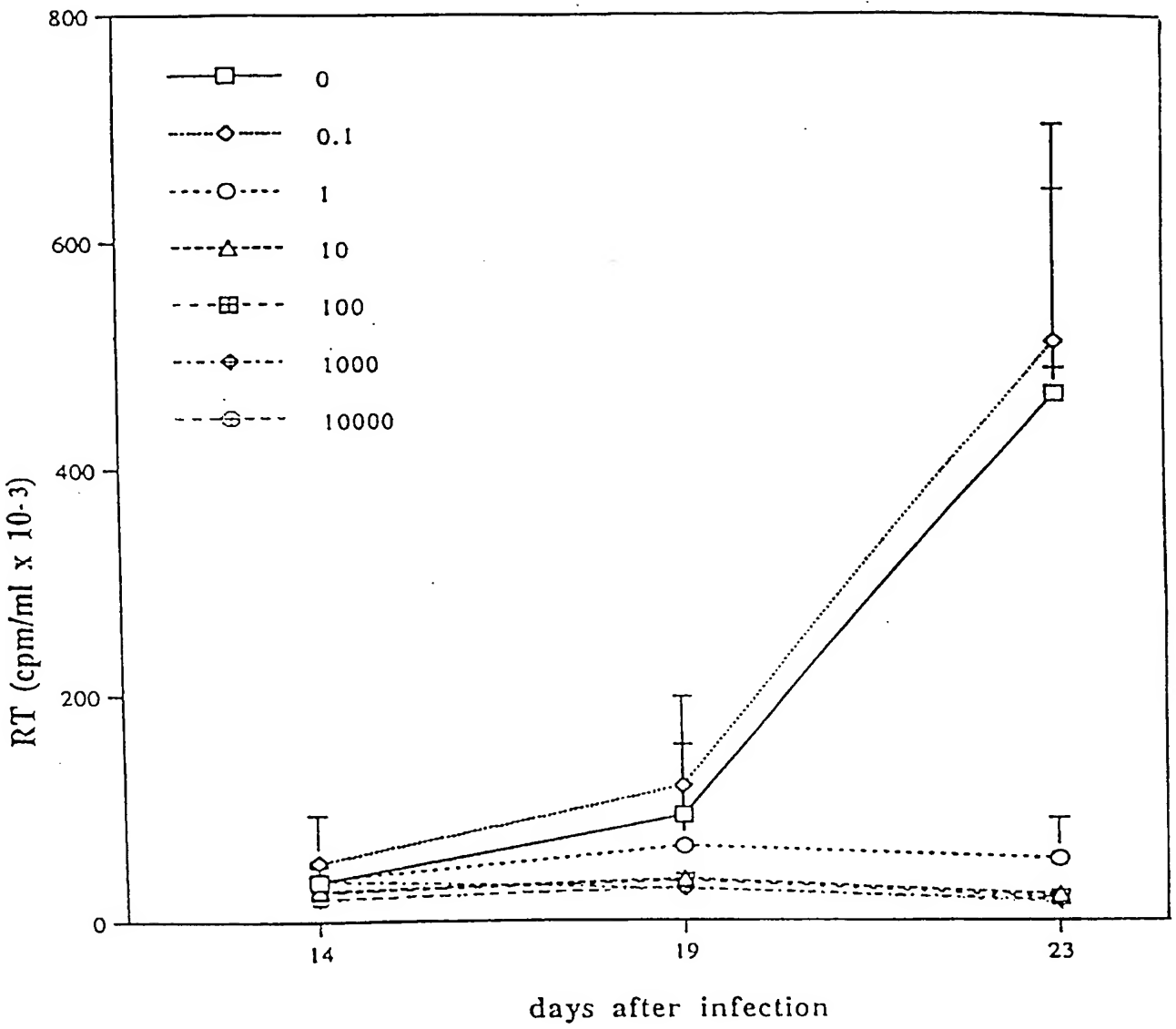

800
600
400
200
0
RT (cpm/ml x 10-3)
0
0.1
1
10
100
1000
10000
14
19
23
days after infection

FIG. 2C

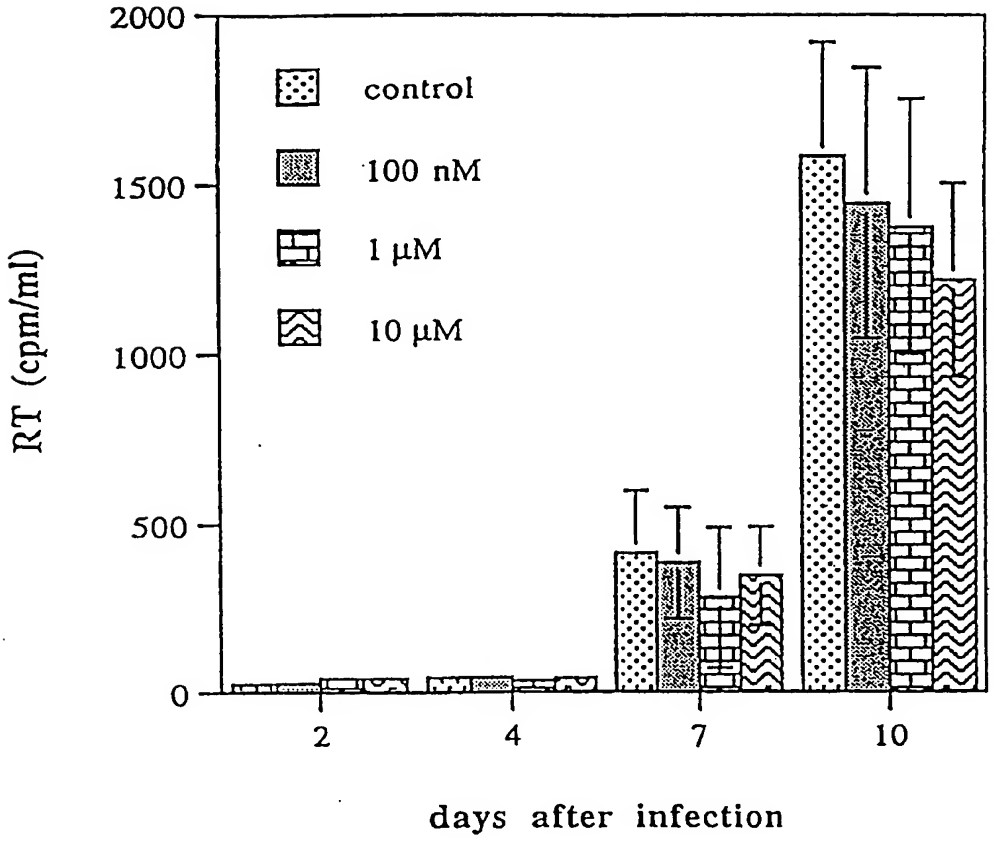

2000
1500
1000
500
0
RT (cpm/ml)
control
100 nM
1 µM
10 µM
2
4
7
10
days after infection

Figure 3A

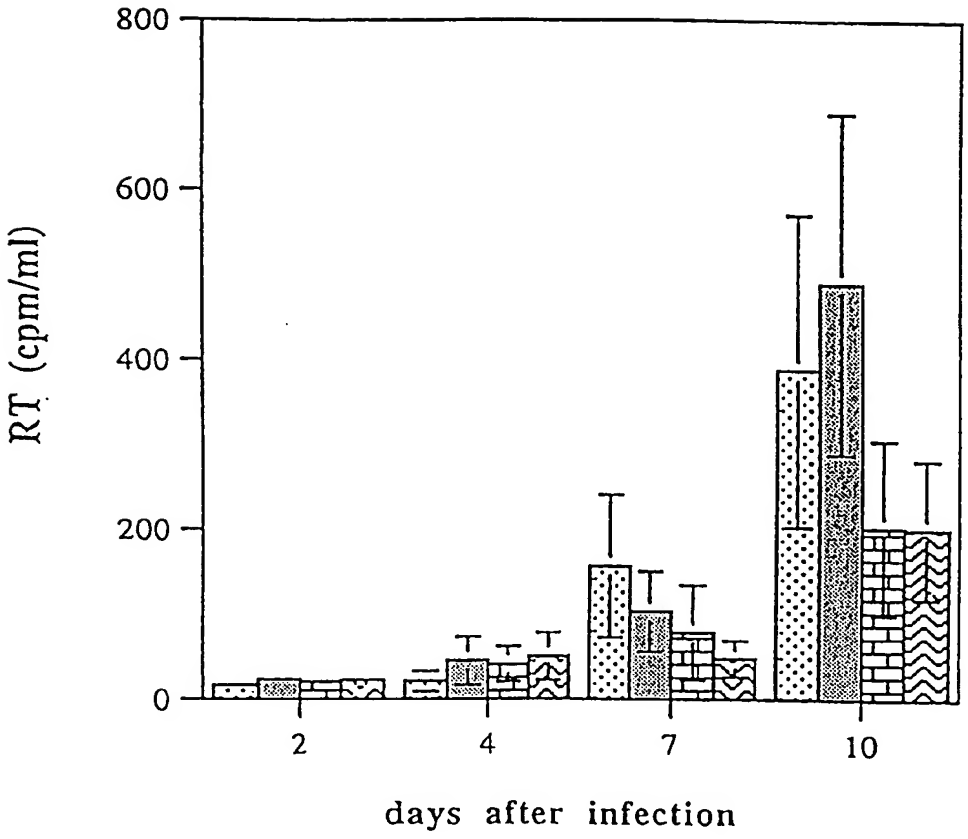


Figure 3B

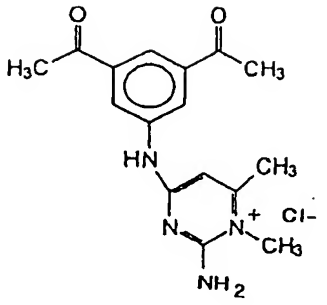


FIG. 4A

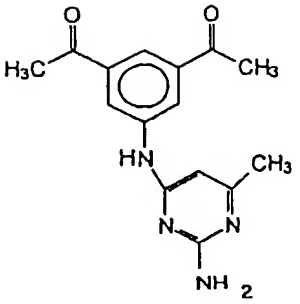


FIG. 4B

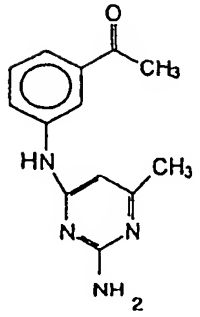


FIG. 4C

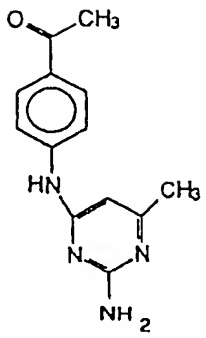


FIG. 4D

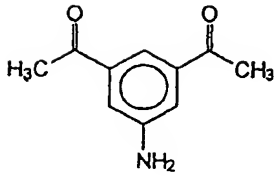


FIG. 4E

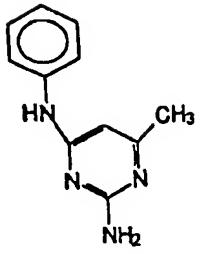


FIG. 4F

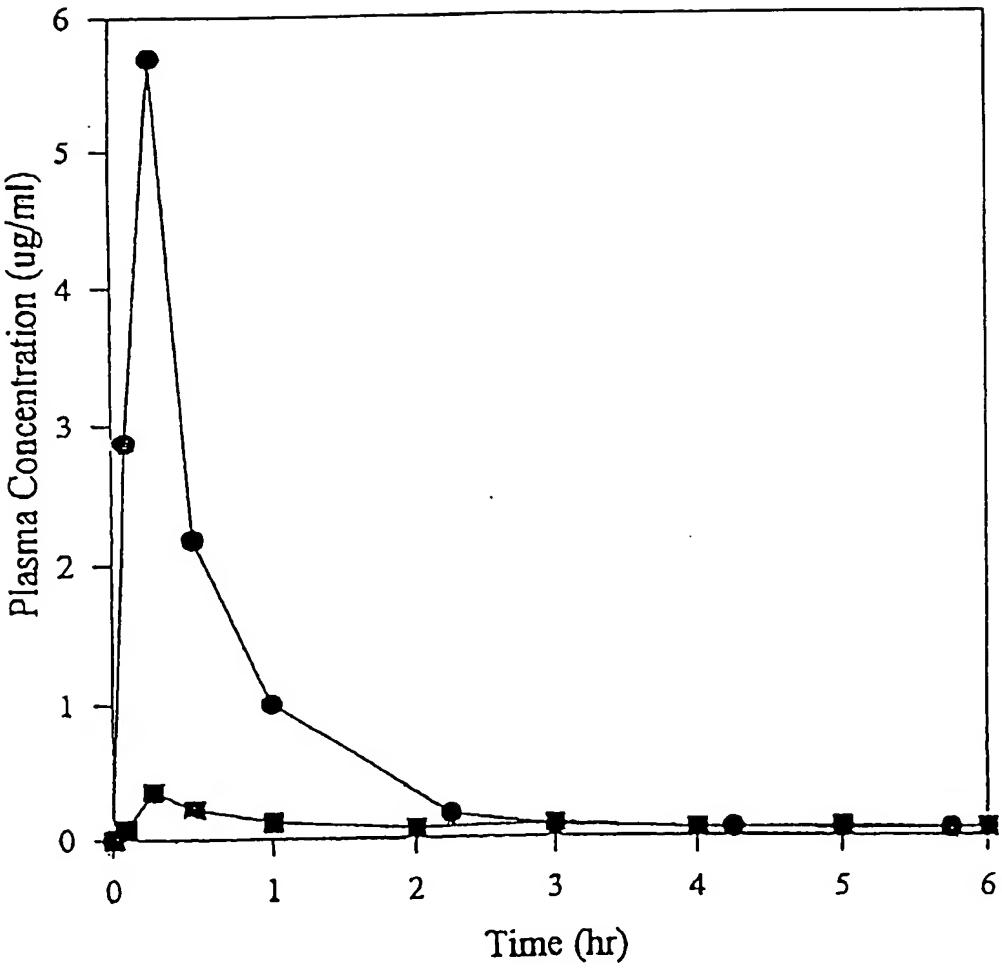


Figure 5

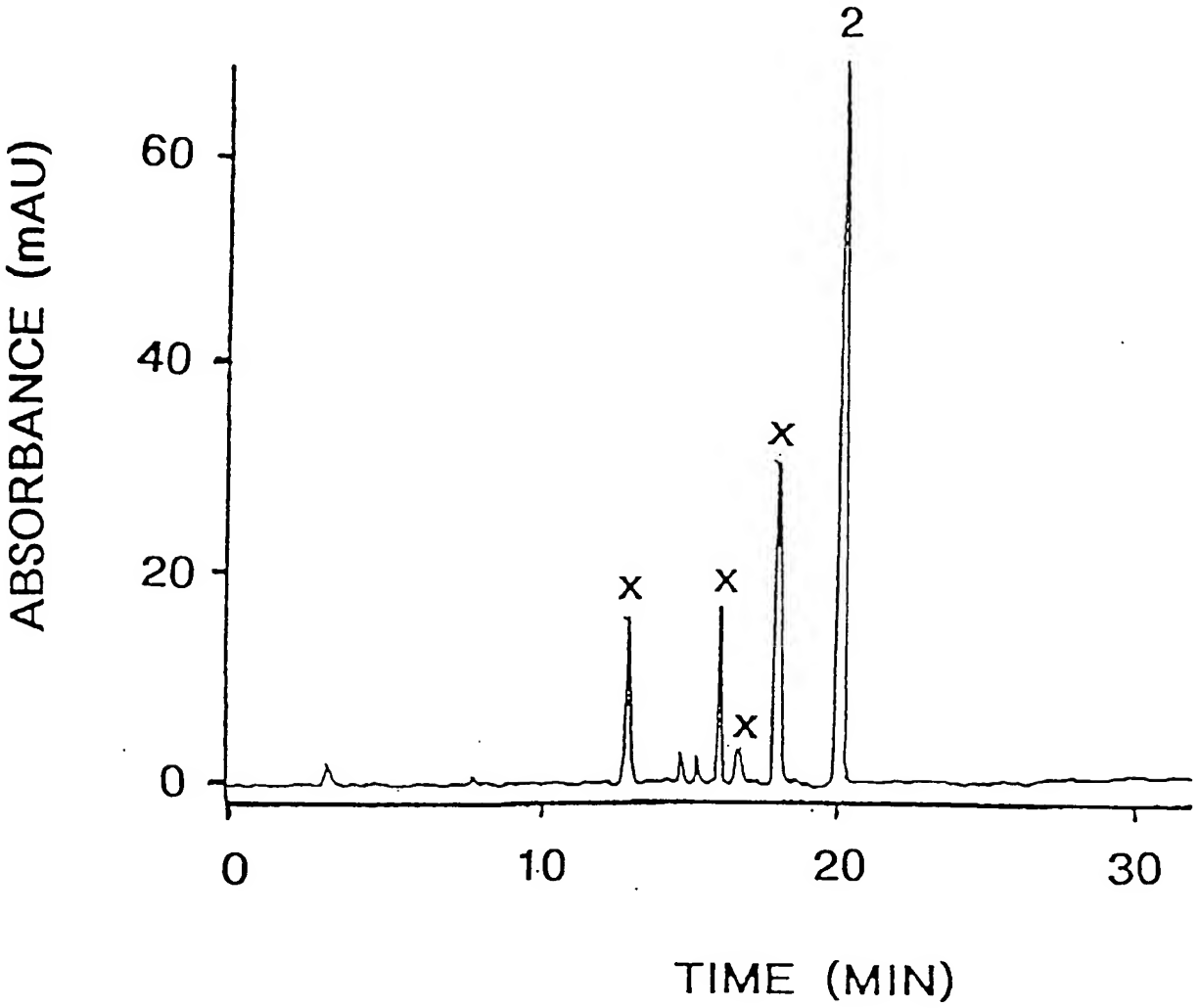


FIG. 6A

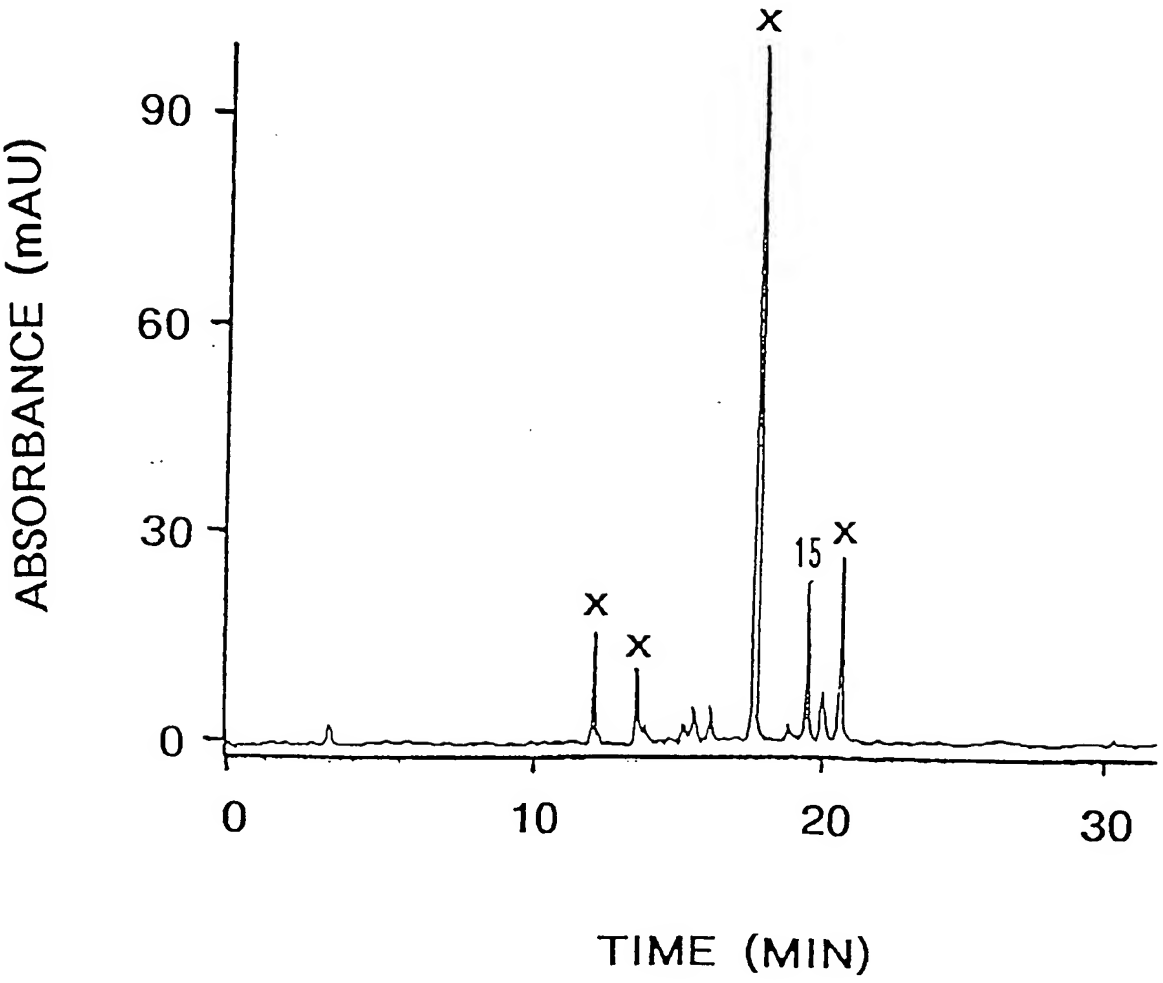


FIG. 6B

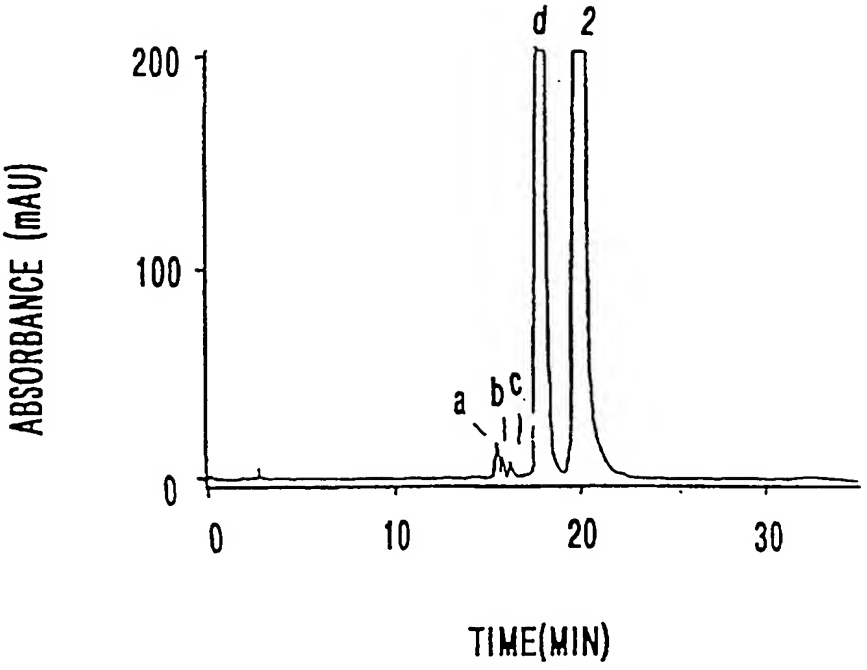

d
2
200
100
0
ABSORBANCE (mAU)
a
b
c
0
10
20
30
TIME(MIN)

FIG. 7A

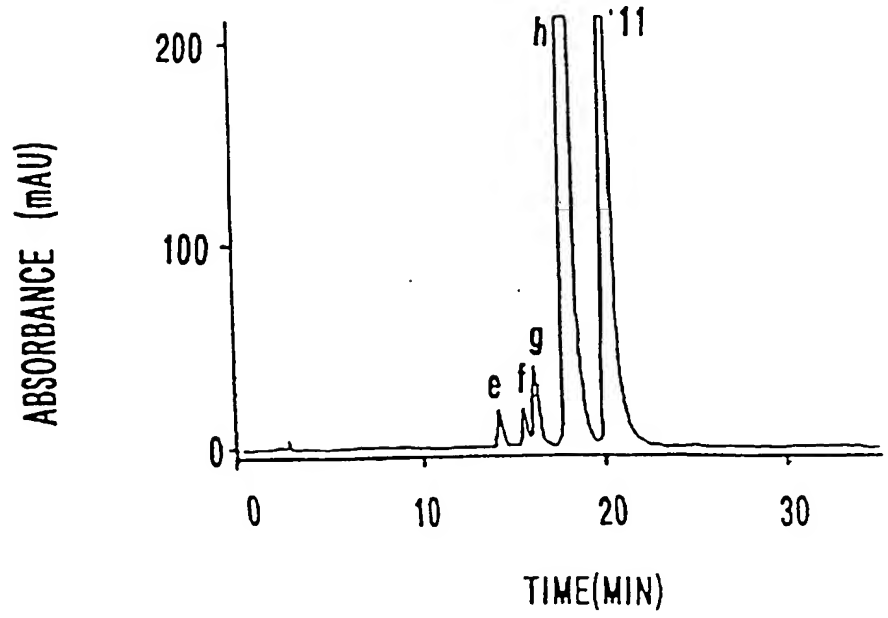

h
11
200
100
0
ABSORBANCE (mAU)
g
e
f
0
10
20
30
TIME(MIN)

FIG. 7B

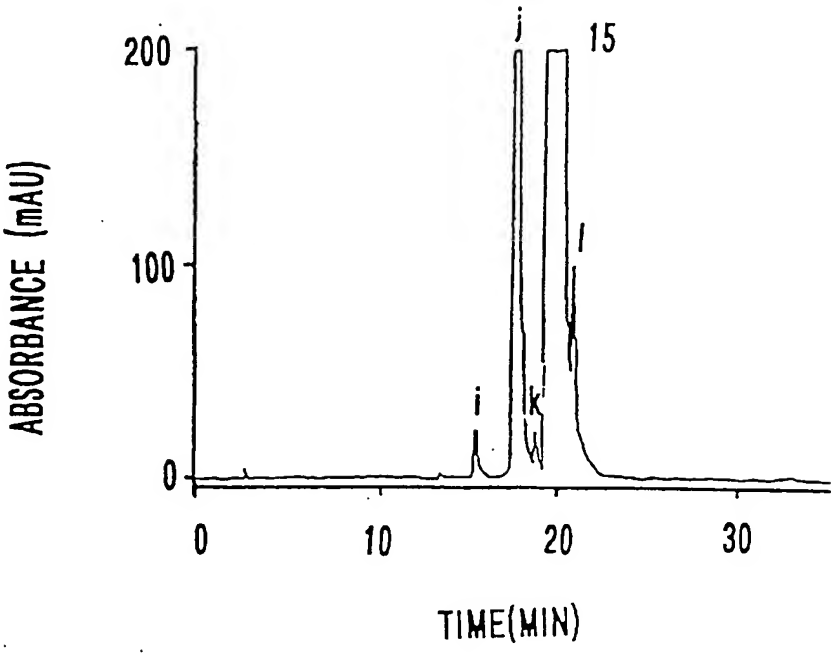

200
100
0
ABSORBANCE (mAU)
0
10
20
30
TIME(MIN)
i
j
k
l
15

FIG. 7C

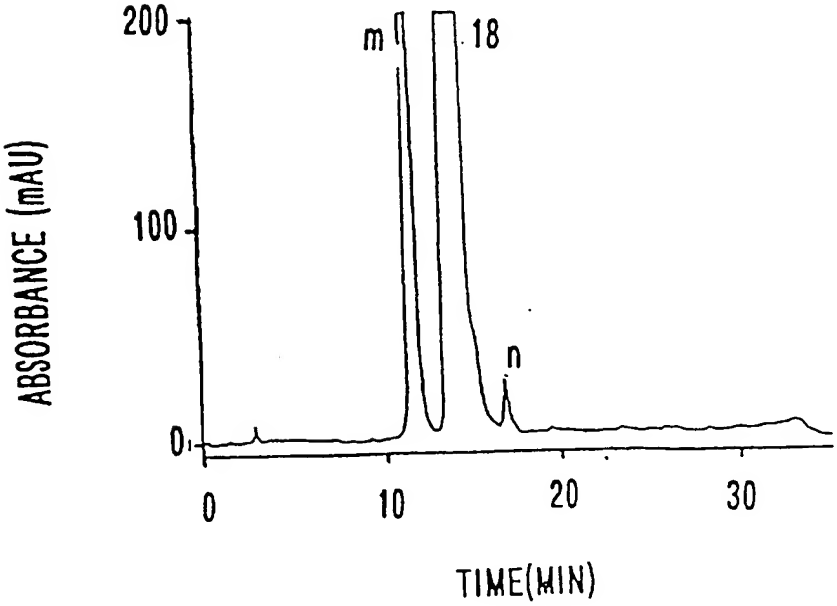

200
100
0
ABSORBANCE (mAU)
0
10
20
30
TIME(MIN)
m
n
18

FIG. 7D

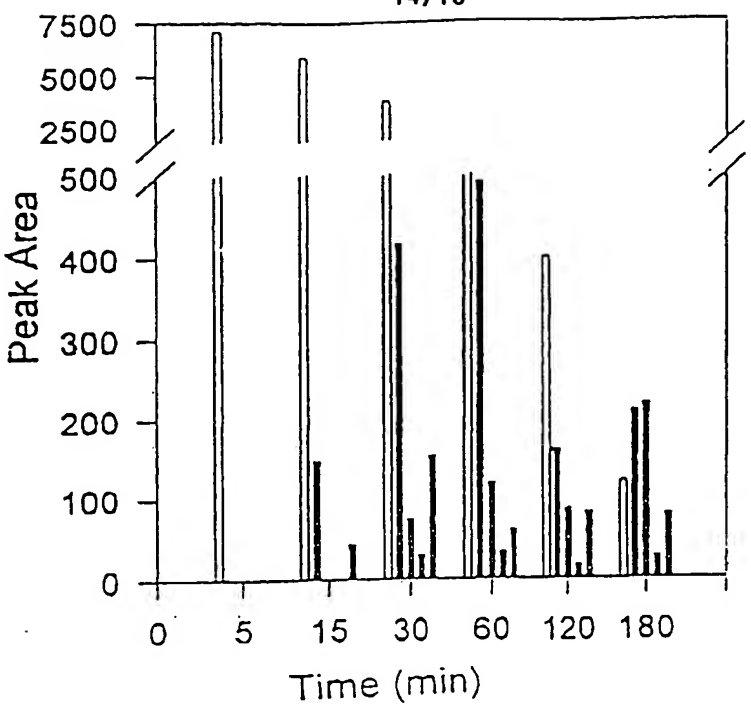

7500
5000
2500
500
400
300
200
100
0
Peak Area
0
5
15
30
60
120
180
Time (min)

FIG. 8A

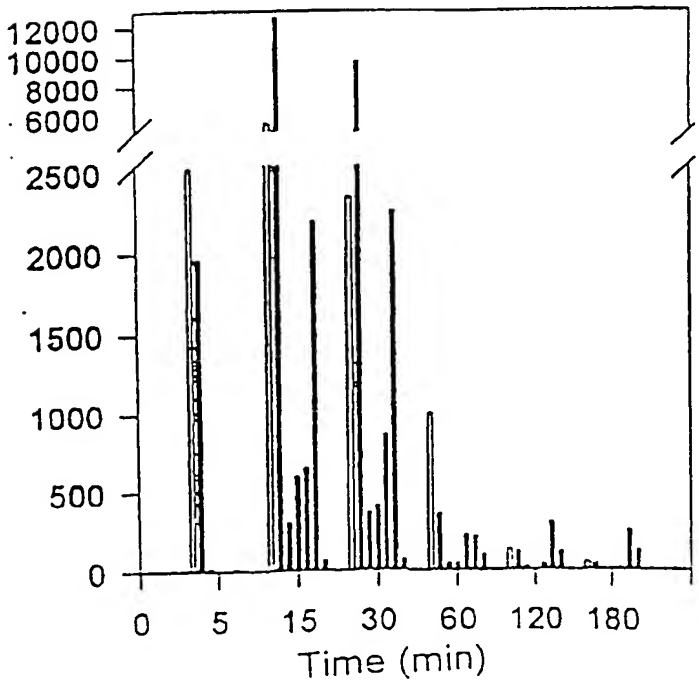

12000
10000
8000
6000
2500
2000
1500
1000
500
0
0
5
15
30
60
120
180
Time (min)

FIG. 8B

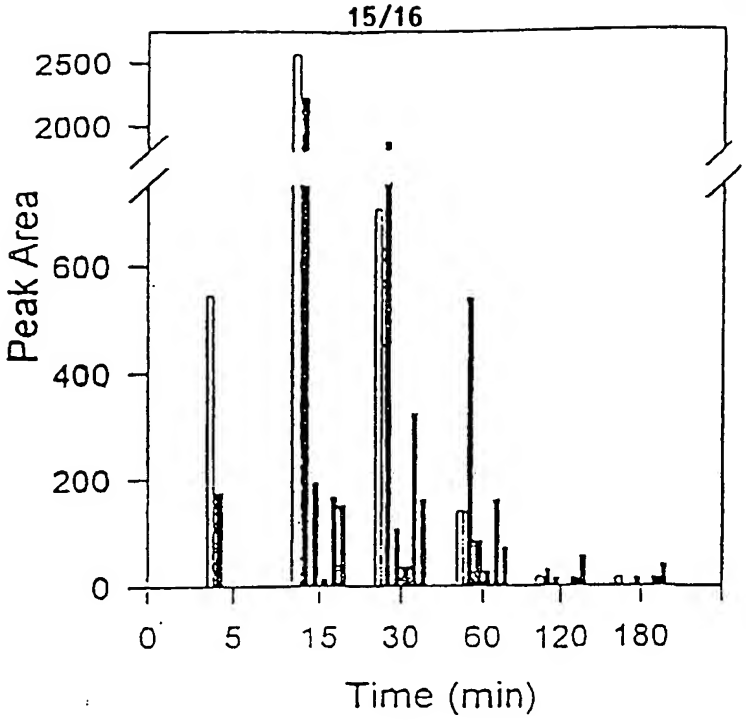

2500
2000
600
400
200
0
Peak Area
0
5
15
30
60
120
180
Time (min)

FIG. 8C

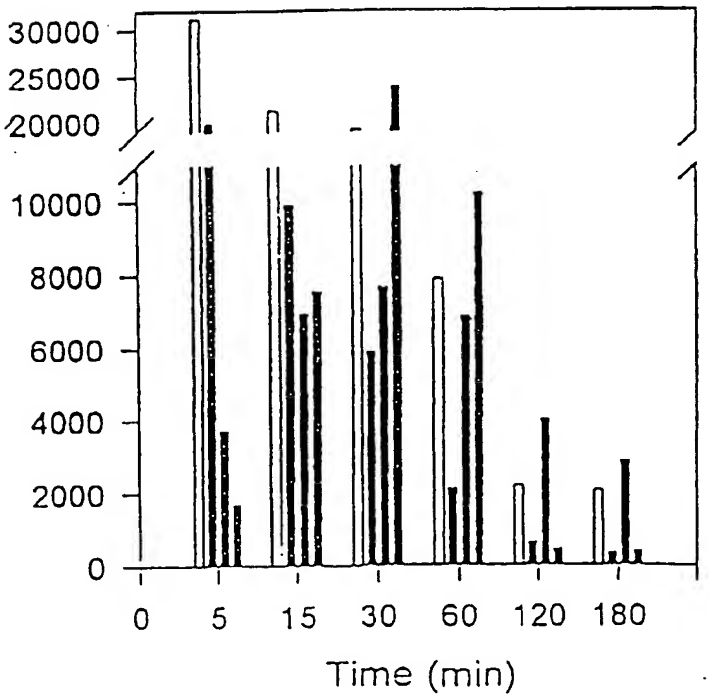

30000
25000
20000
10000
8000
6000
4000
2000
0
0
5
15
30
60
120
180
Time (min)

FIG. 8D

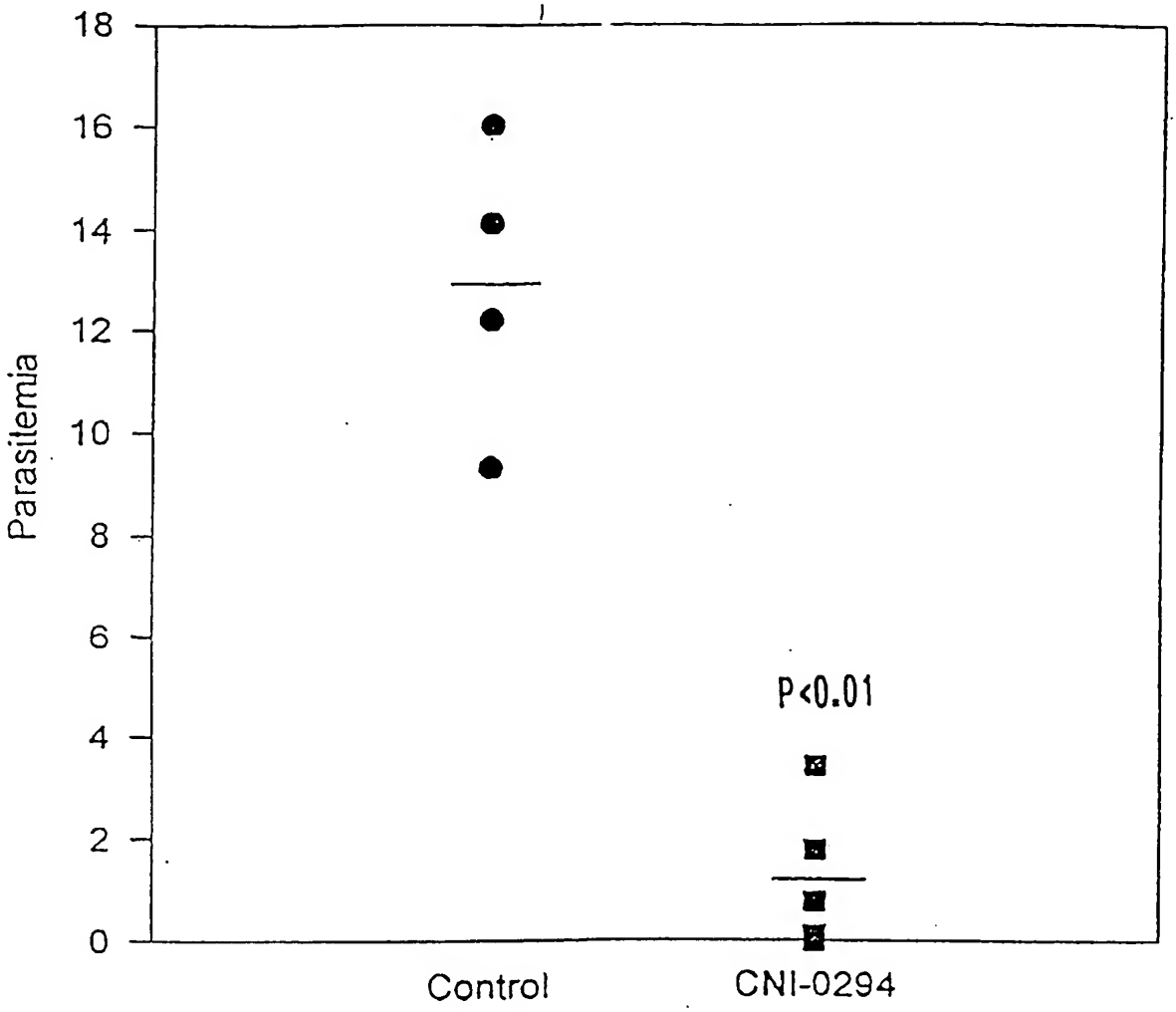


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19071

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07D 239/02; A01N 43/54
US CL :544/323; 514/272
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/323; 514/272

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	BERGER et al. Studies on the pharmacological properties of novel Arylene bis(methylketone) compounds using Solid-phase Extraction and High-performance Liquid Chromatography. Journal of Chromatography B. 11 April 1997, Vol. 691, No. 2, pages 433-440, especially page 434.	1-10 ------ 1-10
X ---- Y	BERGER et al. Antimalarial activity of novel Arylene Bis(methylketone) compounds. The Journal of Infectious Diseases. September 1996, Vol. 174, No. 3, pages 659-662, especially page 660.	1-10 ----- 1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 JANUARY 1998	Date of mailing of the international search report 18 FEB 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer TAMTHOM T. NGO Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*